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THE ANTI-AUTOLYTIC EFFECT OF EPINEPHRINE IN SKELETAL MUSCLE: NON-ADDITIVE PROCESS FOR PRESERVATION OF MEAT[a,b]

C. RADOUCO-THOMAS,* C. LATASTE-DOROLLE,** R. ZENDER,***
R. BUSSET,**** H. M. MEYER**** AND R. F. MOUTON*****
Battelle Memorial Institute, Geneva, Switzerland

(Manuscript received August 16, 1958)

The problem of meat preservation is connected with autolysis and surface microbial contamination phenomena. The main processes being used at present for the purpose of better preservation are essentially the following: cold chain, radio-sterilization, antibiotics, and other chemical substances. All these techniques are generally used post-mortem.

The problem is: (a) to find a process which will counteract autolysis phenomena in muscle and yet not alter or even enhance its organoleptic properties; (b) the process must not be noxious, in any degree, to public health *(4, 5)*.

A number of the properties of epinephrine, which characterize its action at the level of the intermediary metabolism of the muscle *(2)*, has made it possible to meet these requirements and thus to ensure better preservation and a genuine improvement of organoleptic properties, particularly tenderness. It is conceivable that the higher pH—low glucidic and high lactic acid levels—induced by epinephrine in skeletal muscles, might ensure on the one hand an inhibition of the exudative phenomena connected to the denaturation of actomyosine (pH_1) and on the other hand the inhibition of disintegrative proteolytic phenomena which are due to cathepsine activity. Moreover, epinephrine cannot be considered as an "additive product," for it is normally produced and quickly destroyed by the organism. The mechanism of the "anti-autolytic and plasticizer" effect of adrenaline has been indirectly shown by Simone Radouco-Thomas *(7b)* in the study of the effects in the muscle of the antagonist of the adrenergic system.

This paper deals with the effect of ante-mortem administration of epinephrine on metabolism, on the structure of the skeletal muscle stored under aseptic and anaerobic conditions, and with the consequent effects of epinephrine on the preservation and the organoleptic properties of meat.

EXPERIMENTAL METHODS

The skeletal muscles of ante-mortem adrenalized animals have been investigated in parallel with muscles of control animals.

---

[a] Presented in part at the 117th Meeting of the Nutrition Society, London, 1958, and at the 4th International Congress of Biochemistry, Vienna, September, 1958.

[b] This investigation was carried out under the sponsorship of the General Electric Research Laboratories (Schenectady, New York).

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The tests were carried out on two groups of animals: the first group including laboratory animals, i.e. rabbits and rats; the second one consisting of sheep, hogs, heifers and horses considered as slaughter-house animals.

Four hours before killing, extemporaneous 1-epinephrine solution (1‰) was subcutaneously administered to the animal, the chosen dose being generally 250 γ/kg.

Complementary investigations have been conducted on muscles from animals to which epinephrine was given by varying the doses (10 to 1000 γ/kg.) and the time-table (0 to 24 hrs.).

When taking samples of muscle, the utmost aseptic conditions were kept. The excised muscle was generally the *Longissimus dorsi* and sometimes the *psoas.* Rats were rapidly beheaded and the spinal cord was destroyed within the first 30 seconds. Rabbits were stunned and immediately bled by severing the jugular veins.

Sheep and horses were killed by the usual slaughter-house processes. Hogs, after having been submitted to electroshock, were bled and dipped for 3 min. in hot water at 60° C.

The *storage conditions* which are presented in other papers by Zender, Lataste-Dorolle *et al. (8, 9, 10),* consist in enclosing the samples of muscle in special anaerobic tubes (alkaline pyrogallol) and in keeping them in the dark at various temperatures: freezing temperature (4° C.), normal temperature (25° C.) and tropical temperature (38° C.).

The *metabolic modifications* were investigated through the following tests:

pH was directly taken in the muscle and several electrometric measurements (±0.02) were made for each muscle;

Exudative phenomena were quantitatively investigated (ml./100 g. muscle). Some data concerning the constituents of the exudate (K^+/Na^+, proteins and amino acids) and their variations during storage, both in adrenalized and control muscles, have been established by spectrophotometric measurements.

Carbohydrate rates were evaluated on 5 g. homogenates (Waring blender) of frozen muscle in 100 ml. of 5% trichloracetic acid. The homogenate was kept for 10 minutes in a water-bath (100° C.) and filtered; the filtrate was used for quantity evaluation of the glycogen, free hexoses, and lactic acid.

The quantitative evaluation of glycogen was made after precipitation of the glycogen by alcohol, by means of the Zacharias-Dische anthrone method *(3).* An accuracy of ±3.7% for 50 γ and ±7% for 10 γ glycogen was obtained.

The free hexoses were subtracted from the total hexoses having been measured by the same method as applied direct on the trichloracetic acid filtrate.

As regards lactic acid, Barker-Summerson's colorimetric determination *(1)* was chosen (this original method being somewhat modified and its accuracy thereby increased). Beckman's DU spectrophotometer method gives an accuracy of ±3% at an optical density of 0.500.

Glycemia and lactacidemia were determined by the same methods, using the plasma trichloracetic acid filtrate and heparine as anticoagulant.

The *protein and amino acid level* during storage was measured spectrophotometrically at 279 mμ through variation of the tyrosine-tryptophane index.

As previously described *(8, 9),* the muscle proteins were extracted in glycin 1 M NaOH buffered at pH 8.6.

The concentration of the homogenate was 1% for rat muscle and 30% for the other animals: rabbit, sheep, heifer, and hog.

Electrophoretic measurements were made at 4° C., 10 mA for 5,000 seconds.

The *structural modifications* were systematically investigated by interference microscopy, combining morphological examination with microphotography after dissection in the Szent György-Hodge solution. The technique adopted and the nomenclature regarding all stages of degeneracy were given previously *(8),* and complementary data are given in this paper.

Estimation of the *organoleptic properties* was based essentially on the external aspect, the colour, the surface moisture, the "drip," and the flavour of the meat both before and after cooking. The palatability and mainly the tenderness of the cooked, grilled, or roasted meat were statistically evaluated, especially in the case of hog.

Metabolic, structural and organoleptic examinations were made immediately after slaughter and during storage up to several weeks. This allowed us to establish the effect/time and effect/dose relationship.

RESULTS AND DISCUSSION

We now present, both for laboratory and slaughter-house animals, some of the metabolic, structural, and organoleptic modifications in adrenalized and control muscles aseptically excised and stored under various conditions. We have mainly investigated rabbit and hog muscles, studying the various modifications in relation to exudative and disintegrative phenomena. Miscellaneous data concerning other animals, however, are also reported (rat, sheep, ox, etc.).

A. Metabolic modifications

Laboratory animals. As regards laboratory animals, we have systematically experimented on rabbit muscles; some experiments have also been carried out on rats.

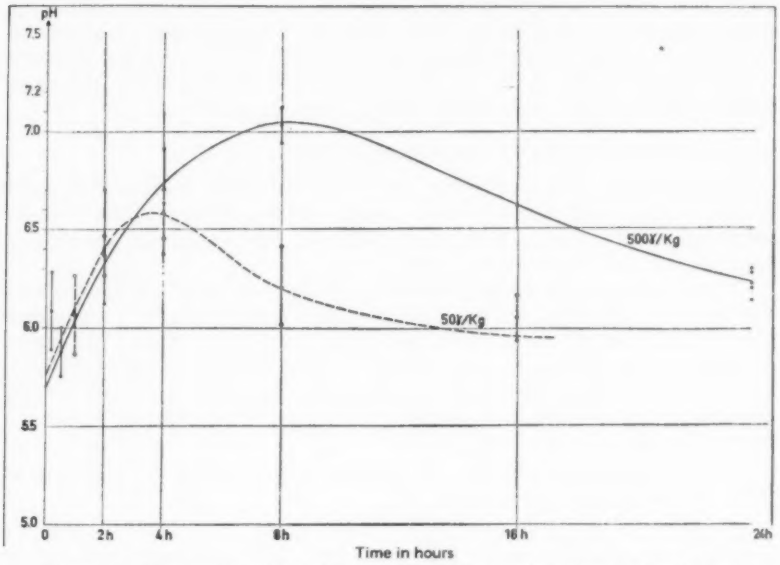


Figure 1. pH values in rabbit adrenalized muscle at various times before killing.

1. *Killing time/effect relationship at a given epinephrine dose in rabbit muscle.*

In order to determine the optimum delay between epinephrine administration and killing, we have selected a most simple and quick effect of epinephrine at the muscle level, i.e. the pH drop inhibition checked *in vitro*.

Epinephrine was administered at various times ranging from 10 to 24 hrs. before killing. These experiments were carried out for 50 γ and 500 γ/kg. epinephrine ante-mortem. The measurements were systematically made at the time of the pH stabilization, i.e. 20 hrs. after killing.

Figure 1 illustrates the variation of pH. First of all, we observe that the pH in control muscles drops towards 5.7. To get the best epinephrine inhibition with the 50 γ/kg. dose, a period of administration taking place 3 hrs.

prior to killing appears suitable as the pH is then blocked at 6.6. With the 500 γ/kg. dose, the period between 4 and 8 hrs. should be chosen according to the respective stabilization at 6.7 and 7.

Thus, a delay of 3 to 5 hrs. between the epinephrine injection time and the killing time appears to be optimum, as it ensures a maximum efficacy for low and medium doses of epinephrine.

2. *Dose/effect relationship at a given time of epinephrine administration (4 hours before killing) for rabbit muscle.*

In order to determine the range of the active epinephrine doses and the optimum active dose, we have studied the effect of 8 various doses (0 to 1000 γ/kg.) administered 4 hrs. prior to killing.

The experiment was carried out on 24 animals, divided in 8 groups of 3 animals, in order to check the 0, 10, 25, 50, 100, 250, 500 and 1000 γ/kg. epinephrine doses, respectively. Two, out of the 6 *Longissimus dorsi* muscles removed in each group, were stored for 20 hrs. at 20° C.[c] while the 4 others were kept for 14 days at 38° C. The pH variation, the exudation, and some protidic aspects were examined in the two storage conditions.

(a) *Early effects within the first twenty hours at 20° C.*

(i) *Variation of pH.* Figure 2 shows the pH drop inhibition through gradual increased epinephrine doses. After a 20 hr. storage of the controls we observe a pH drop in them from 7.2 to 5.6, whereas, in the adrenalized samples, the pH drop is prevented.

A relation seems to occur between the inhibition of the pH drop and the dose of epinephrine. Figure 2 shows that 10 γ/kg. epinephrine represents the minimum active dose, while quite a strong action appears for about 50 γ/kg. Along with higher doses (>100 γ), the pH varies slightly (6.8–7.0).

According to the inter-animal variability, we may infer that doses greater than 50 γ/kg. might be suitable to prevent the pH from dropping below the 6.5 to 7 range.

(ii) *Exudation phenomena.* No exudation was visible after 20 hrs. at 20° C. in the adrenalized samples.

(iii) *Variations of glycine-soluble proteins and amino acids.* After 24 hours' storage (25° C.) the "T. T." index [d] for G. S. protein is up to 26.6 as well for controls as for adrenalized samples in the case of doses lower than 100 γ/kg. epinephrine. For higher doses, this index rises slightly (Figure 2).

The amino acid "T.T." index was identical in controls and adrenalized muscles, whatever the administered dose (Figure 2).

(b) *Subsequent effect after a 14 days' storage at 38° C.*

(i) *Variation of pH.* It appears from Figure 3 that within a long period of storage, the inhibitive epinephrine effect on pH drop is still appreciable and is related to the increasing doses. However, with respect to a slightly higher pH in the controls (Figure 3) stored during the same time, the slope appears less steep than after 24 hours. As we have already seen above for short storage, the 50 γ/kg. dose appears to have quite a significant effect. The

[c] That is, 20° C. ± 2° C.

[d] Tyrosine—Tryptophane index (see above in Experimental Methods).

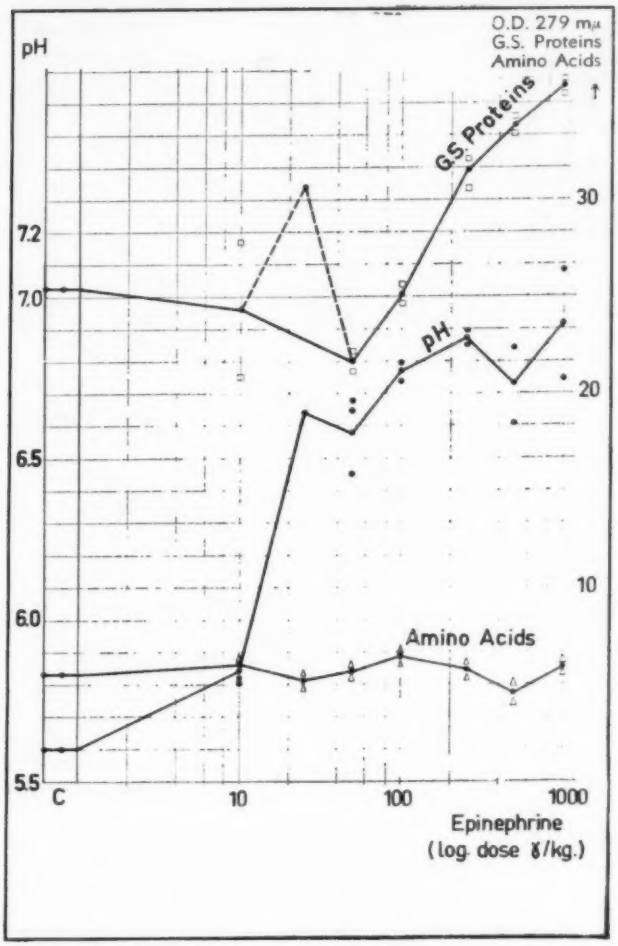


Figure 2. Effect of increasing doses of epinephrine on some metabolic aspects of rabbit muscle (20 hours/25° C.).

curve following doses higher than 50 γ/kg. flattens out between the values of pH 6 and 7.0.

(ii) *Exudation phenomena.* In control muscles the exudation reached an average of 7 ml. for each sample. Even the slightest dose of 10 γ/kg. inhibited significantly the exudation phenomena (2.5 ml.). Higher doses gave a plateau between the 100 γ/kg. dose (1 ml.) and the 1000 γ/kg. dose which seemed to stop exudation completely.*

* In Figure 3, the quantitative evaluations have been made in weight percentages.

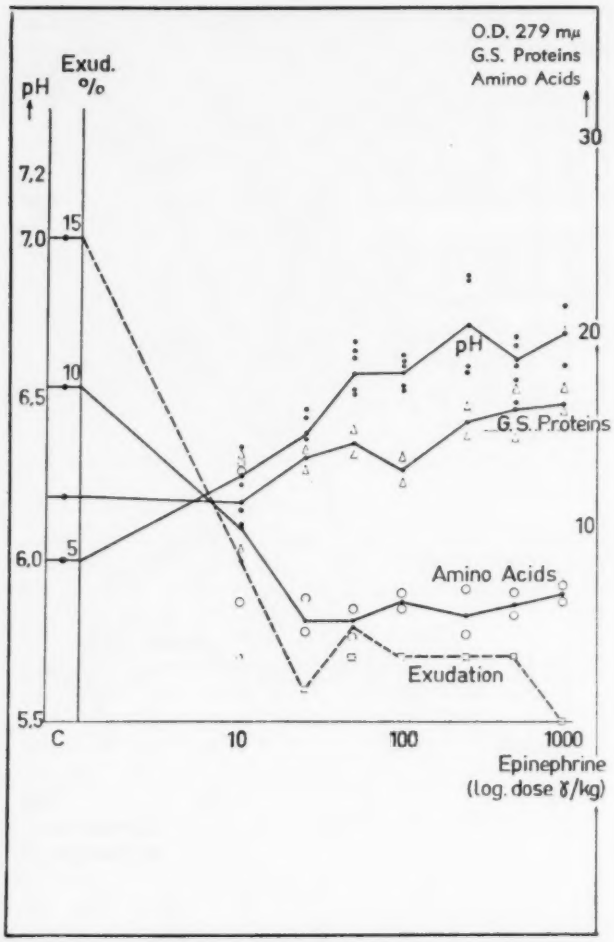


Figure 3. Effect of increasing doses of epinephrine on some metabolic aspects of the rabbit muscle stored for 14 days at 38° C.

(iii) *Variation of glycine-soluble proteins and amino acids rates.* Compared with the controls, the G. S. protein level seemed to be slightly enhanced with increased epinephrine doses. The amino acids level was quite high in the controls (Figure 3), and the inihibition by epinephrine of the amino acids production was already observable for the 10 γ dose and remained on a fairly constant level from the 50 γ dose up to 1000 γ dose.

On the whole, it appears that epinephrine inhibits the pH drop, the exudation, and the production of amino acids. This activity can be observed with a dose of 10 γ/kg. and becomes clearly visible from 50 γ/kg. on. In order thus to be at the effective dose level for all animals, and to avoid too

strong an hyperadrenergic syndrome, we have chosen a medium standard dose of 250 γ/kg.

3. *The epinephrine effect at predetermined dose and killing time (250 γ/kg., 4 hours before killing) in rabbit muscle.*

For both the suitable dose of 250 γ/kg. and the suitable delay before killing, the same metabolic aspects as above, pH, exudation, G. S. protein and amino acids were tested for various periods of storage, in control and adrenalized muscles. Some aspects of the carbohydrate metabolism have also been investigated.

(i) *Variation of pH.* The variation of pH was checked *in vivo* and *in vitro*. *In vivo,* the pH was verified on anaesthesized animals found to be about 7.2 as well in controls as in adrenalized animals; during the *first 8 hrs.' storage* at 25° C. (*see* Figure 4) the pH appeared to drop quickly to 5.75 in

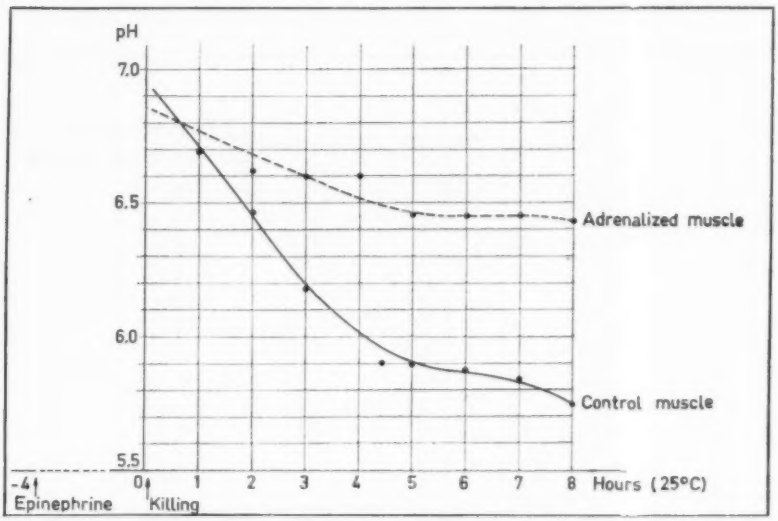


Figure 4. Variation of pH in muscle excised from 250 γ/kg. adrenalized (4 hours prior to killing) and control rabbits during the first 8 hours at 25° C.

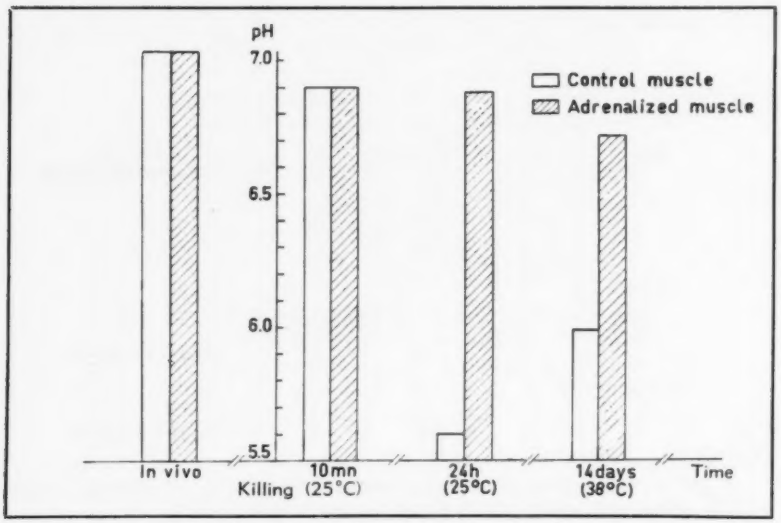
the controls and was stabilized at quite a high level—6.5—after a slight decrease during the first 4 hrs., in the adrenalized samples. After *a period of 14 days' storage* at 38° C., both in the control and the adrenalized animals, pH values appeared unchanged, about pH 5.5 in the control, and still at high pH (6.9) in the adrenalized muscles. Figure 5 illustrates the blocking effect of epinephrine on pH variation until the 14th day of storage.

(ii) *Exudation phenomenon.* Our previous data *(8)* is confirmed through the present controls. A very strong exudation is observed. The exudation flow (ml./day) runs through 3 successive phases: there is an initial rapid flow within the first 24 hrs. (about 5 ml./day); the second phase lasts until the 7th or 12th day (about 0.8 cc./day); from then on there is a slight gradual decrease until the 50th day (about 0.2 ml./day).

The composition of the exudate has been investigated. Its protein content increases up to high values, whereas its amino acid level remains low. Parallel to this, electrolyte variations are observed. K concentration increases gradually while Na concentration is at a low and constant level. In the very first exudation phase K^+/Na^+ ratio is about 10.

In the *adrenalized samples* the exudative phenomenon is inhibited in the first early phase and strongly reduced (80% of the control exudation) after 14 days' storage at 38° C. (Figure 3).

(iii) *Some aspects of the protide metabolism.* The *T. T. amino acid indexes* for control and adrenalized rabbit muscle extracts are evaluated at about 9. O. D. in the initial period. A similar slight decrease is observed for both samples 20 hrs. later, but after 15 days' storage the amino acid level in the controls rises up to 17. O. D. while it remains very near its initial value in the adrenalized samples.



Figrue 5. Variation of pH in muscle excised from 250 γ/kg. adrenalized (4 hours prior to killing) and control rabbits.

The *T. T. protein indexes* for control and adrenalized glycine-soluble extracts are similarly evaluated. Being around 13. O. D. in the inital period, they both rise up to 100% of their original amount in the first 20 hrs. (25.5 O.D. and 28.8 O.D., respectively). Later, after 14 days' storage—at 38° C.—the two samples have returned to the initial values, i.e.: 11.6 and 13.9, respectively.

The *electrophoretic pattern of G. S. protein* diagrams of controls and adrenalized samples were interpreted after 20 hrs. and 14 days' storage at 38° C.

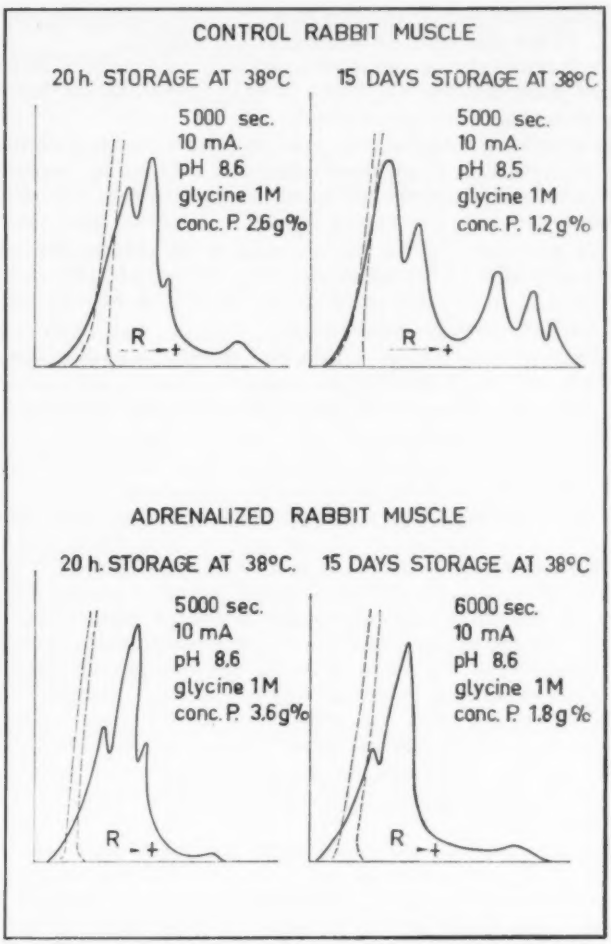


Figure 6. Electrophoretic diagram of G. S. proteins from "adrenalized" and non-adrenalized muscle after a 20 hr. and 14 days' storage at 38° C.

Fourteenth-day storage control diagrams compared to the 24-hr. ones bring out characteristic new protein sub-unit peaks. As regards the "adrenalin sample" diagram, compared to the control diagram, a slight difference appears at the beginning. We will try to explain this difference in a subsequent report.

Further, as opposed to the control diagram data, no new sub-unit peaks, characteristic of proteolysis, appear on the "adrenalin" electrophoretic pattern (see Figure 6).

(iv) *Some aspects of the carbohydrate metabolism.* Muscle carbohydrate constituents, such as glycogen, free hexoses and lactic acid have been

examined in control and adrenalized rabbit muscles immediately after killing (5 min.). At the same time we investigated rat muscles.

Figure 7 shows that a strong glycogenolysis has occurred in the 250 γ adrenalized rabbit muscles. Compared to those of the controls, the values of free hexoses and lactic acid are lowered.

These investigations have been completed by the control of the hyperglycemia and the hyperlactacidemia classically induced by epinephrine.

The following *miscellaneous data* concern muscles from 250 γ/kg. adrenalized rats:

(i) *pH variation.* In the non-adrenalized rat muscle, the initial pH has the same value as in rabbit and hog, but it dropped steeply from 6.8 down to 5.2. In the adrenalized muscle, the pH, throughout the 14 days' storage, remained between 6.5 and 6.8.

(ii) *Exudation phenomena.* In the case of rat *Longissimus dorsi,* which represents a very small material, the exudative phenomena is not easy to evaluate. However it was noted that, compared to the adrenalized samples, the controls are very moist.

(iii) *Some aspects of protide metabolism.* Data obtained with rat control muscle extracts show that the G. S. proteins rise about 15 (O. D. 279 mμ) in the first period and remain at this constant level throughout the 14 days' storage.

In the adrenalized muscle, the G. S. protein went up to 17.3 O.D. after killing, and 18.2 O.D. after 24 hrs. Later, it seemed to us that the variation of the G. S. protein adrenalized rat muscle did not appear significant and that during the storage period, the G. S. proteins increased paradoxically.

The variations of the amino acids for the control and adrenalized rat muscles appeared as insignificant as they are for G. S. proteins, i.e. similar values in control and adrenalized muscles immediately after killing, 24 hrs. later, followed by the similar paradoxal increase on the 14th day.

These apparent variations during long storage could probably be accounted for by some analytic circumstances perhaps related to the glycine solubilization ability.[1]

(iv) *Some aspects of the carbohydrate metabolism have been tested on rats.* The glycogen, free hexoses and lactic acid have also been investigated under the same conditions as those indicated for rabbit. A marked glycogenolysis and a low level in free hexoses and lactic acid can be observed (Figure 7, right hand).

Slaughter-house animals

1. *Epinephrine effect on hog muscle.*

As regards slaughter-house animals, we have systematically investigated *hog muscle.* We performed experiments, using adrenalized animals and controls, for a period of 35 days' storage at 38° C. under aseptic, anaerobic

[1] When investigating rat muscle, only a small quantity (i.e. 1 g.) of *Longissimus dorsi* can easily be obtained for submission to 100 ml. glycine solvent. For the same type of muscle, but in rabbit, it is easy to find 30 g. for the same 100 ml. glycine extraction. For muscle homogenates of 1% and 30%, the tyrosine-tryptophane index at 279 mμ increases for rabbit from 13,020 to 22,000 O.D. and for rat from 14,220 to 34,550 O.D. Further investigations were performed, and the subject will subsequently be discussed elsewhere.

conditions *(8)*. The medium standard dose of epinephrine (250 γ/kg.) was applied 4 hours prior to slaughtering.

The variation of pH, the exudation, and some modifications in the protide metabolism were tested (Figure 8).

(i) *Variation of pH.* As in the case of rabbit muscles, the *control hog muscle* shows an initial pH of about 6.8 which drops to 5.6 in the rigor phase. Later on, the pH increases slightly up to 5.8–6.0—probably due to gradual autolysis occurring during the 35 days' storage and especially to autolysis of the fats which release fatty acids.

In the *adrenalized hog muscle,* the pH remains about 6.5 during the entire period of storage (35 days).

(ii) *Exudation phenomena.* Hog meat is known to be strongly exudative. In the control samples, the total volume of exudate was evaluated up to 11% (in weight) the first day and reached 15–16% at the end of the first fortnight. Epinephrine seems to be able to counteract this rather high exudation: exudation is negligible until the 7th day (1%) and rises to about 4% in weight on the 35th day.

(iii) *Some aspects in the protide metabolism.* The initial values of the G. S. proteins appeared a little higher in the adrenalized than in the control samples. During 35 days' storage, the G. S. proteins content decreases, whereas the amino acids increase. In Figure 8 the respective curves of controls sand adrenalized animals have similar patterns: in both muscles, the G. S. proteins are lowered to nearly half of their initial content; the amino acids increased very slightly. The convergence of the curves is more marked for the controls.

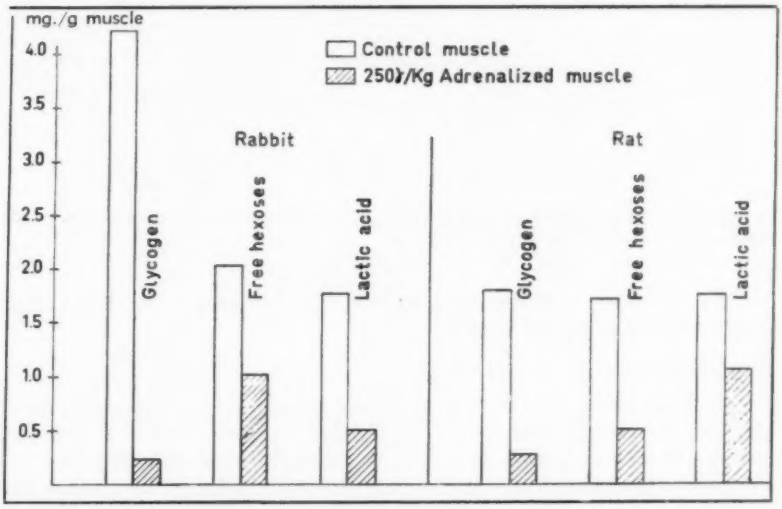


Figure 7. Values of glycogen, free hexoses and lactic acid in muscle excised from 250 γ/kg. adrenalized and control rabbit and rat.

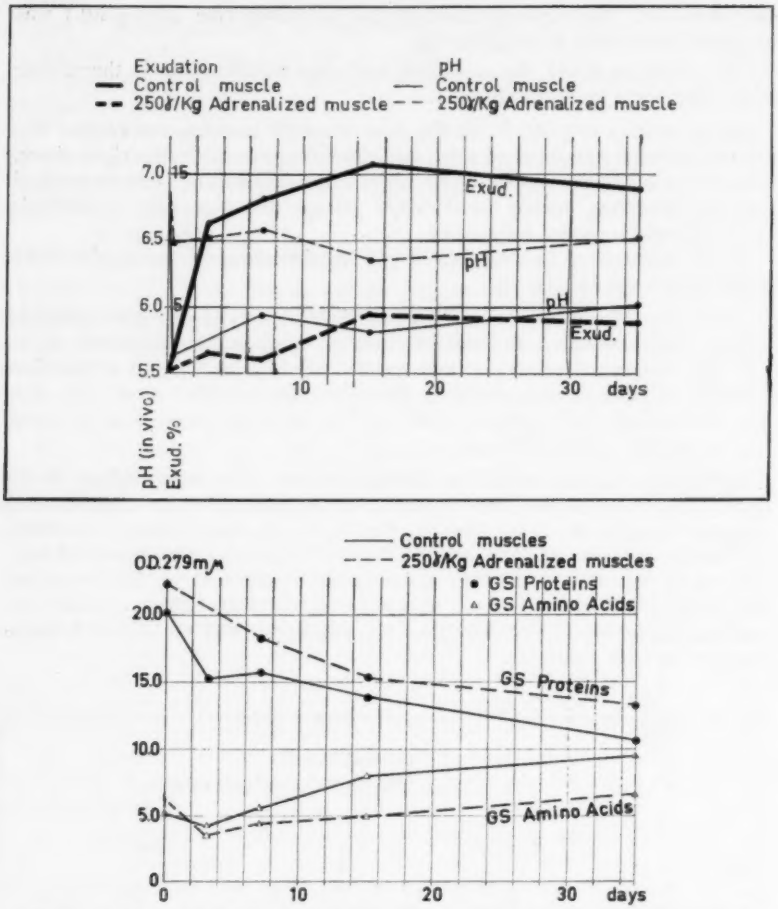


Figure 8. Some metabolic modifications in muscle removed from adrenalized and control hogs (35 days' storage at 38° C.).

The following *miscellaneous data* concern muscles from 250 γ/kg. adrenalized sheep, ox and horse:

2. *Epinephrine effect on sheep muscle.*

(i) *Variation of pH.* The pH drop in control sheep muscles appears slower and less pronounced than it is in rabbit, rat, and hog. After 20 hrs. and during a long storage of 50 days at 35° C., the pH remained around 6.0.

In the adrenalized sheep muscles, the pH drops slightly to an average pH of 6.45 and remains between 6.4 and 6.7 throughout the storage of 50 days at 38° C.

(ii) *Exudation phenomena.* With regard to this phenomenon, no difference can be detected between controls and tests after 6 days. After a longer storage of 50 days at 38° C., the total volume of the control exudate is ten times as high as the volume of the adrenalized samples.

(iii) *Some aspects of protein metabolism.* No striking difference appears between adrenalized and non-adrenalized sheep muscles after the quite long 50 days' storage at 38° C. The data are similar to that described for the hog.

3. *Miscellaneous data for ox and horse.*

The pH of ox muscle *in vitro* appears to be between 5.5 and 6.0 in the control muscle, and 6.3 and 6.7 in the adrenalized one, but we should mention that for exceptional reasons the measurements were made with universal pH paper dipped in the homogenized muscle.

As regards horse, the number of experiments seems insufficient, as yet, to draw conclusive results.

B. Structural modifications

In a previous work *(8)* we combined the biochemical tests with investigations on the intimate structure modulus in phase contrast appearance. A terminology[g] is attempted to classify the main morphological aspects of the fibre itself and those of its band pattern.

Laboratory animals. As regards laboratory animals the epinephrine effect on muscle structure has been investigated on rats and rabbits at various times:

1. *Epinephrine effect observed on rat muscles at time of excision.*

The structural aspect of the skeletal muscle has been studied in interference microscopy on controls and "adrenalized"[h] rat muscles immediately after killing in the very first period of the pre-rigor phase (15 min. post-mortem).

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[g] The special symbols used here are as follows:

Macroscopic data, at time of dissection
T = tenderness (flaccid, firm, hardened meat)
Ex. = extensibility
D = dilaceration into separate fibres

Microscopic positive phase contrast appearances

(a) For the fibres:

Turgescency
Elasticity, linear walls . . .
Stiffening, indented walls . . .
Shortening-stretching, more or less strong
Flexuring, more or less acute accordion shape
Splitting, transversal or longitudinal,
shape of *"chess-board"* for strong transv. splitting
shape of *"fans"* for long. splitting
Disruption
Flattening
Degeneracy (inapparent walls)

(b) *For the band-pattern*

"Hyphened striation" = aspect of dotted lines

"Plexiframe"

"Granular striation" = aspect of scattered spots

[h] Under our standard condition stated above (250 γ/kg. 4 hrs. prior to killing).

At that time the two samples, controls and epinephrine, present the same macroscopic characters of tenderness and extensibility. For both samples, dilaceration into separate fibres is quite easy in spite of their high elasticity.

Microscopically, the data are similar: the fibres are generally long and straight, and the classical microscopic rigor band pattern, regular shortened spaces between striations, does not appear.

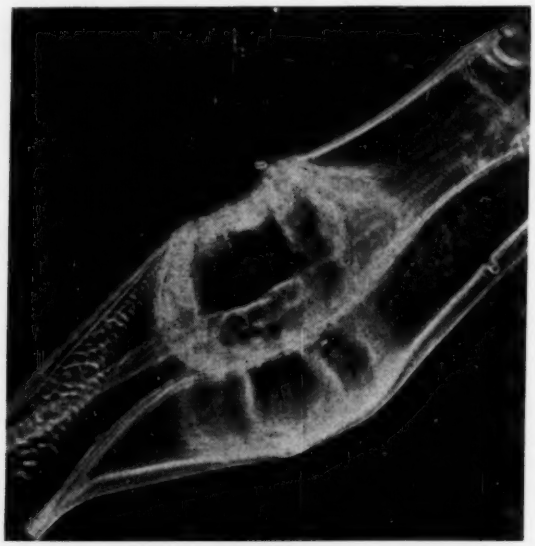
The main feature is an alternate acute shortening-stretching, as well in controls as in "adrenalized" muscles.

Another feature seems to be the same in the controls and the epinephrine slides: the isotropic band (low, dense, non-birefringent) is seen regularly dotted, either drawn similarly to the same bands previously observed in aseptic autolysis in the course of storage, or distorted and showing the feature of a network [1] or "plexiframe."

According to the above microscopic features, it is possible that the morphological appearance of rat muscle, whether adrenalized or not, excised just post-mortem and submitted as soon as possible to dissection and interference illumination under physiological conditions, could suggest a microscopic morphological similarity with the unknown *in vivo*-like state. This problem will be dealt with in further work by investigating under new interference and electron microscopy techniques.

The next picture (Figure 9), robot camera shot, shows the feature of strong stretching-shortening of the rat fibres, whether adrenalized or not, and the subsequent morphological band pattern modifications.

[1] So-called "hyphened striation."



x 820

Figure 9. Positive phase-contrast appearance of shortening-stretching and plexiframe bands on non-adrenalized and adrenalized rat muscle at the time of excision.

TABLE 1

Macroscopic morphological aspects of non-adrenalized and 50γ and 250γ/kg. adrenalized rabbit fibres 20 hr./25° C. and 14 days/38° C. storage

Experim. conditions	Tests	Storage	
		20 hr. at 25° C.	14 days at 38° C.
"Non-adrenalized muscle" (Controls)	T	Hardened meat	Excessive tenderness
	Ex.	Non-extensible (rigor)	Disrupted
	D	Not easy to dissect: the contracted fibres are disrupted by the needles into short *non-elastic* fibres	The meat smashes under the needles and disintegrates into short soft pieces, while SGH liquid becomes latex-like
"Adrenalized muscle" 50 γ°kg.	T	Firm	Quite tender
	Ex.	Quite extensible	Still extensible
	D	Quite easy to separate into rather long elastic fibres	Quite easy to dissect into bundles of long fibres; clear SGH suspension
"Adrenalized muscle" 250 γ°kg.	T	Firm flexible	Flaccid
	Ex.	Extensible	Still extensible
	D	Easy to dilacerate into long separated elastic fibres	Easy to dissect into long elastic elements; clear SGH suspension

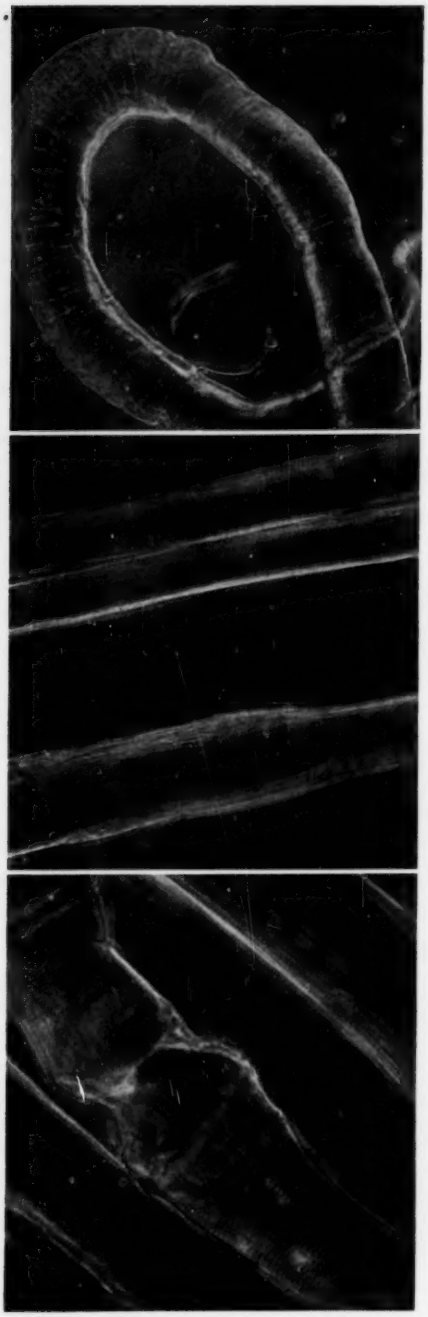
T = tenderness
Ex. = extensibility
D = observation in time of dilaceration
SGH = Szent-György-Hodge isotonic liquid:
Szent-György 50% glycerol : 1 part
Hodge solution KCl. 1 M-Mg.Cl$_2$. 001 M: 1 part

2. Epinephrine effect observed in rabbit muscles after 20 hours (25° C.)

Rabbit muscle has been systematically studied at rigor stage from both macroscopic and microscopic points of view.

(a) *Macroscopic data.* Table 1 shows the various aspects of the controls compared to those of the adrenalized muscles (50 γ and 250 γ/kg.) as regards their respective tenderness, extensibility, and ability to be dilacerated into separate fibres.

The characteristics of the control meat are those of rigor mortis, according to the hardness, inextensibility and difficulty of dissection. Both 50 γ and 250 γ/kg. epinephrine doses seem to depress some aspects of the rigor phenomena, even though the whole carcass and the large piece cut off have the usual rigor firmness. Under the dissection needles, the adrenalized material, compared to the non-adrenalized one, really appears less hard, still



(a)

Controls

Indented walls
Regularly shortly spaced bands
(x 608)

(b)

50 γ/kg. Adrenalized

Linear walls
Slight shortening-stretching
(x 608)

(c)

250 γ/kg. Adrenalized

Turgescency
Shortening-stretching
plexiframe bands
(x 656)

Figure 10. General structural aspect and band pattern on controls (a), 50 γ/kg. (b), and 250 γ/kg. (c) adrenalized rabbit muscles in short storage (20 hr./25° C.).

flexible, and easy to dilacerate into separate elastic fibres. The 250 γ/kg. epinephrine rabbit skeletal isolated fibres seem in particular to be as extensible and easy to dilacerate after 20 hr. at 25° C. as were the rat fibres at the time of excision.

(b) *Microscopic data.* Table 2 summarizes the phase contrast appearances, and Figure 10 shows the main aspects for the same controls and adrenalized samples. It is clear that:

(i) The *controls* show the typical "rigor mortis" aspects of the skeletal structure: flexuring, indented walls, and regular spaces between continuous bands.

TABLE 2

Microscopic morphological aspects of non-adrenalized and 50 γ and 250 γ/kg. rabbit muscles under positive phase contrast 20 hr./25° C. and 14 days/38° C. storage

Experim. conditions	Tests	Storage	
		20 hr. at 25° C.	14 days at 38° C.
"Non-adrenalized muscle" Controls	General aspect	Turgescency Rather long straight or flexured fibres (indented walls)	Loss of turgescency (flattening) Disruption (chess-board) Areas of total degeneracy 2 : 1 (200%)
	Band pattern	Classical band pattern = regular *shortly spaced* striations	Disintegr. of band pattern: Intact fibres = 2.5% Hyphened fibres = 11.5% Granular fibres = 86.0%
"Adrenalized Muscle" 50 γ/kg.	General aspect	Turgescency Quite long fibres (predominance of straight ones) Features of soft shortening-ning-stretching	A little less turgescency Quite long straight and flexured fibres Some features of shortening-stretching Few areas of total degeneracy (2%)
	Band pattern	Generally typical band pattern and sometimes aspects of hyphened or "plexiframe" striation	Generally well-kept classical band pattern: Intact fibres = 68.0% Hyphened fibres + plexiframe = 31.5% Granular fibres = 10.5%
"Adrenalized muscle" 250 γ/kg.	General aspect	Turgescency Predominant long straight fibres (linear walls) Frequent features of shortening-stretching (more pronounced than in 50 γ/kg. sample)	Turgescency Long straight fibres (linear walls) (very few flexured fibres) No degeneracy Some features of shortening-stretching less pronounced than in the first 24 hr.
	Band pattern	Typical, largely spaced striations, or features of hyphened and "plexiframe" dark bands	Well kept band pattern: Intact fibres = 85 % Hyphened fibres + plexiframe = 55.5% Granular fibres = 5.0%

(ii) The *adrenalized* samples show all the features described above for fresh "post-vital" muscle despite the time of storage (20 hrs.): linear walls, slendering, shortening-stretching, hyphened and plexiframe bands.

According to the dose of epinephrine, we might say that the dose of 50 γ/kg. is capable of minimizing the typical regular rigor shortening, and that in the case of the higher dose (250 γ/kg.) the feature of rabbit skeletal fibre is mainly characterized by the alternate shortening-stretching shapes previously observed in the immediate post-vital phase. Such a similarity between the microscopic appearance of both the controls and adrenalized samples observed immediately post-mortem and the microscopic aspect of only the adrenalized samples after 20 hrs. storage, could allow the inference that an ante-mortem epinephrine administration (250 γ/kg.) would be able to maintain the skeletal fibre with an appearance similar to that of a pre-rigor state and perhaps in a "vivo-like" one.

3. *Epinephrine effect observed in rabbit muscles after a 14 days' storage at 38° C.*

The effect of given doses of 50 γ and 250 γ/kg. epinephrine on rabbit *Longissimus dorsi* has been studied.

The macroscopic and microscopic data are brought together in Table 2, and Figure 11 shows the essential graphic features.

Macroscopically there is no doubt that, despite the aseptic experimental conditions, the meat disintegrates in the *control samples* stored for 14 days at 38° C.

On the contrary, the structural integrity is maintained in the epinephrine samples during the same period of storage. It seems clear, even macroscopically, that the aseptic autolysis, as previously described, is counteracted to some extent by the 50 γ/kg. dose of epinephrine and in a striking way by 250 γ/kg.

Microscopically, under the interference microscope, the degeneration in the *controls* is well demonstrated by the strong structural modifications observed, i.e. loss of turgescency, various splitting and disruptions of the fibre, alteration of the bands, and extended areas of total degeneracy (50%).

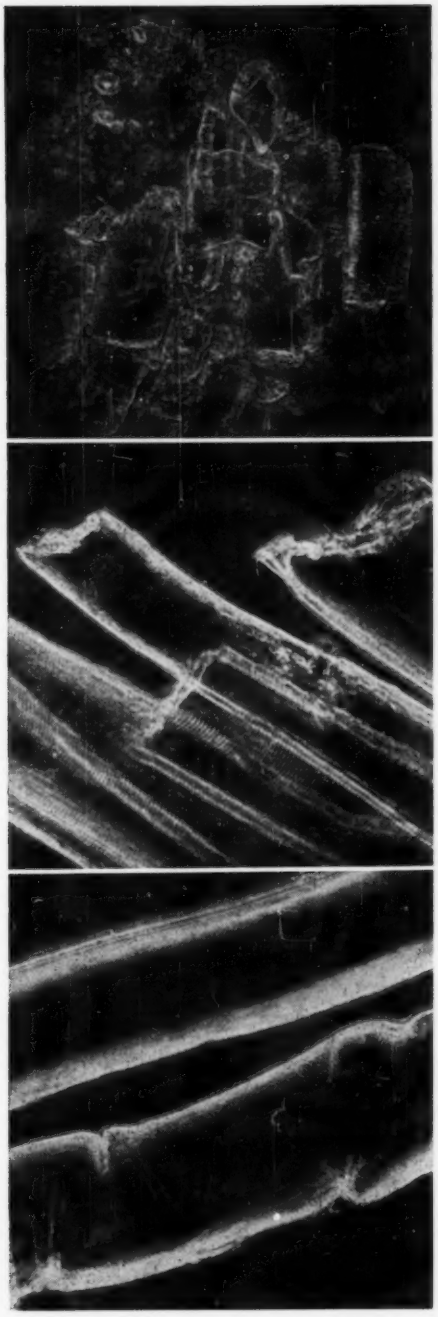
On the other hand, in the epinephrine samples, the microscopic aspects are conserved, i.e. turgescency, and predominantly long straight fibres, with frequent shapes of shortening-stretching, are seen. However, we must say that on epinephrine slides, the features of stretching-shortening appear less frequently and are less obvious after the quite long storage of 14 days at 38° C. than in the first 20 hrs.

It is suggested that the specific epinephrine effect on muscle structure would be reduced during long storage.

Figure 11 illustrates the substantial differences between the two materials, adrenalized or non-adrenalized, after a 14 days' storage at tropical temperature.

The effect of increasing doses of epinephrine (10γ up to 1000 γ/kg.) on rabbit muscle structure has also been systematically investigated under our standard conditions of epinephrine administration and storage.

The data are reported according to the logarithmic graph shown in Figure 12, percentages of the long straight fibres and of the hyphened-plexiframe



(a)

Controls

Disintegrated fibres and
myofibrils
(latex-like SGH liquid)
(x 608)

(b)

50 γ/kg. Adrenalized

Slight turgescency
Alteration of the bands
(SGH clear)
(x 656)

(c)

250 γ/kg. Adrenalized

Distortion—Plexiframe striation
(x 656)

Figure 11. General structural aspect of band pattern on controls (a), 50 γ/kg. (b), and 250 γ/kg. (c) adrenalized rabbit muscles after a fortnight storage at 38° C.

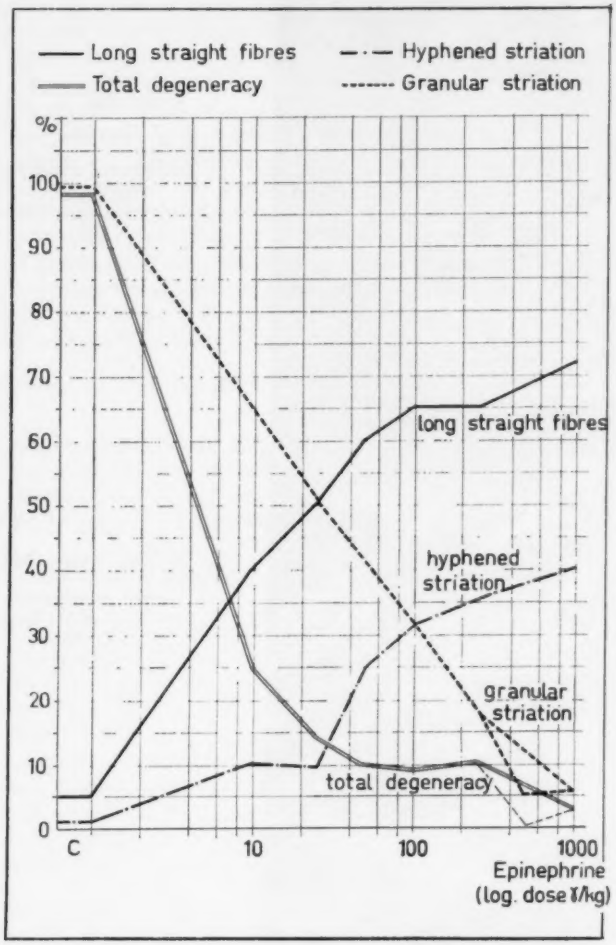


Figure 12. Effect of increasing doses of epinephrine on rabbit muscle structure (14 days at 38° C.).

striations increase with the increasing doses of epinephrine, whereas the various signs of degeneracy inside and outside the fibre decrease markedly. Doses of only 100 γ/kg. epinephrine appear very efficient for the inhibition of the structural disintegration during 14 days' storage at 38° C.

Slaughter-house animals. As regards slaughter-house animals, the epinephrine effect on muscle structure has been investigated mainly after storage up to several weeks at 38° C., on pork and mutton. Some data concerning the beef and the horse are also reported.

1. *Epinephrine effect on hog muscle.*

We have experimented under the standard conditions referred to above on two series of samples from 250 γ/kg. adrenalized and control animals.

(i) *Macroscopic data.* A special difference in the outward aspect is at once pointed out as regards colouration, which is more dense pink in the adrenalized samples. Some discrete hemorrhage suffusions are visible on the surface, and the muscle seems to be in a state of "twitching."

(ii) *Microscopically,* under the disection needles the *control* meat is very tender and easy to dilacerate into short fibres. The S. G. H. liquid is somewhat latescent. The "adrenalized" fibres can be separated into longer elements that are still flexible; their suspension in the S. G. H. liquid looks clear.

Under interference microscopy, the main specific epinephrine features appearing are the shortening-stretching and the hyphened-plexiframe striation.

The graphs in Figure 13 give the quantitative data for intact straight fibres and for the different stages of degeneracy inside and outside the fibres for the two samples respectively—controls and adrenalized—until the 35th day.

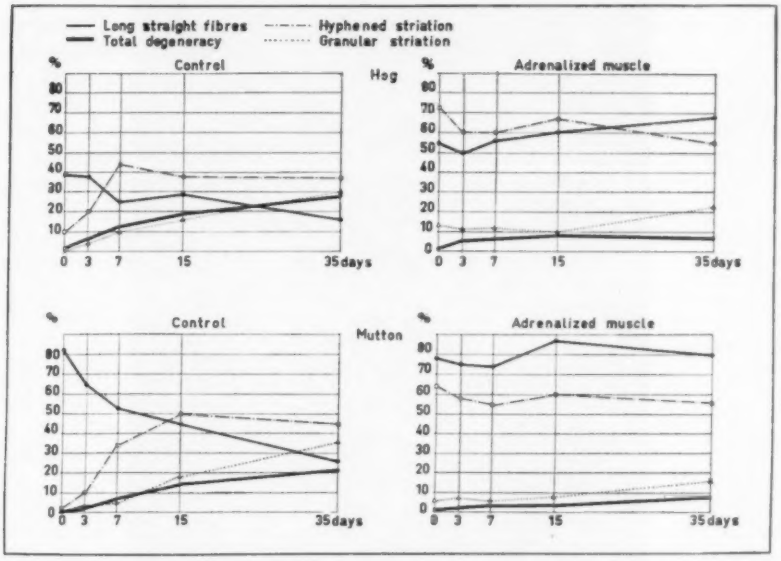


Figure 13. Epinephrine inhibitor effect on aseptic autolytic structural disintegration in hog and mutton muscle (35 days at 38° C.).

During storage of the adrenalized muscle, the percentage of the straight muscle fibres remains high and the percentage of hyphened plexiframe is raised; also the various signs of degeneracy are lowered.

However, under the same standard conditions, it should be noted that the hog meat control disintegrates slower than does rabbit; on the 35th day storage at 38° C., the percentage of total degeneracy is only 28% of, whereas it was nearly 100% in rabbits on the 14th day (Figure 12).

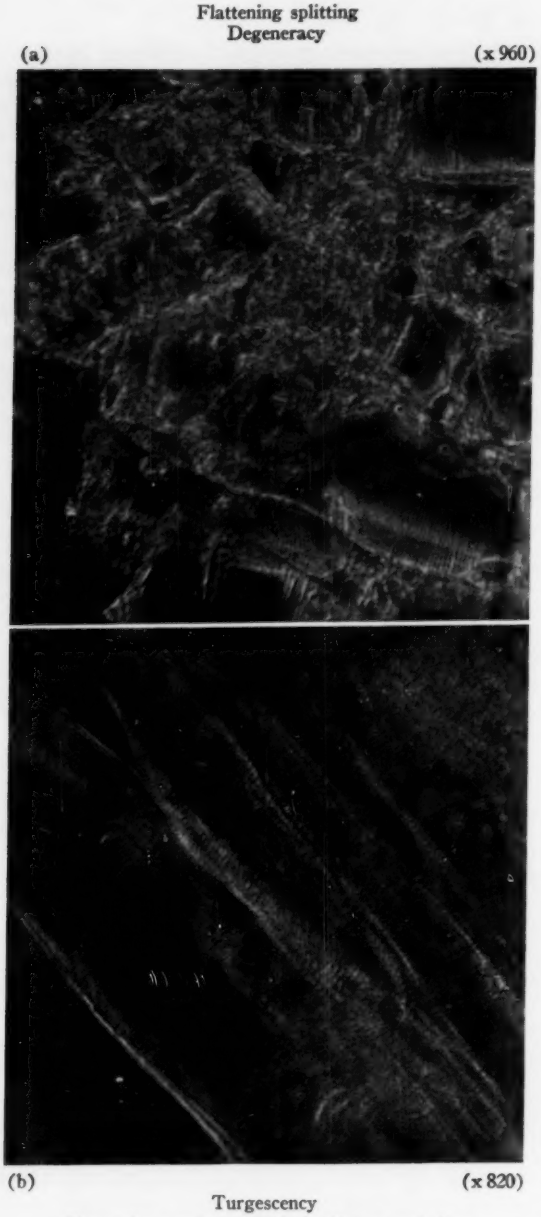


Figure 14. Structural aspect and band pattern of controls (a), and 250 γ/kg. (b) adrenalized hog muscle after 35 days/38° C. storage.

Figure 14 shows the important difference between the two samples, and indicates the improved appearance of the adrenalized samples after a 35 days' storage at tropical temperature.

It should be noted that muscle slides from adrenalized hog, fresh or stored, present frequent features of "exploded fibres," and even "free myofibrils" in the shape of gerbs and rosaces.

Such features can probably be related to the very high count of hyphened-plexiframe striations obtained in hog. They are comparable to the features encountered after long aseptic autolysis in various control animals *(8, 10)*. Yet, rosaces although free of myofibrils and hyphened-plexiframe striation in fresh adrenalized hog do not have exactly the same appearance as the same figures met with in controls. They keep their refringency and the SGH liquid around them is clear.

Experiments have been carried out with samples from fresh adrenalized hog during the first 20 hrs., in order to investigate the possibility of a mechanical longitudinal distintegration effect of epinephrine in the hog fibre.

Figure 15 gives the "phase contrast appearance" results of these investigations, together with similar appearances at the 35th day at 38° C. in non-adrenalized hog.

It is clear that a 250 γ/kg. epinephrine dose immediately induces in hog a special "interfibrillar" splitting, which we call the "adrenalin primary splitting." By analogy, we call the special plexiframe striation that is visible immediately post-mortem, the "primary hyphened striation," as against the "secondary hyphened striation," for which autolysis is known to be responsible.

We suggest that a special action of epinephrine in hog, a "primary interfibrillar splitting," could play its part in the improvement of tenderness of this meat, as hereafter discussed.

2. *Epinephrine effect on sheep muscle.*

In our experiments, the aseptic autolysis of sheep, previously described *(8)*, appears to be undoubtedly counteracted by the epinephrine administered in accordance with our technique.

In Figure 13, the main quantitative data of this inhibitive action in sheep are assembled opposite those of hog meat for the same conditions of storage (35 days at 38° C.). Contrary to the non-adrenalized samples, both the "adrenalized" sheep and hog meat show very weak signs of degeneracy, inside and outside the fibres, which are—predominantly—long continuous fibres with alternate stretching-shortenings.

Concerning the "primary hyphened striation" the level is high in sheep meat, but a little lower than in hog meat. The count throughout 35 days at 38° C. is, as in hog, quite constant.

With regard to the "primary interfibrillar splitting," no such feature is encountered in "adrenalin" sheep slides, either at the beginning of storage or at the end of it (35th day).

3. *Miscellaneous data for ox and horse.*

Some rapid observations carried out under phase contrast reveal the same features as proof of the epinephrine inhibitive autolysis effect: turgescency

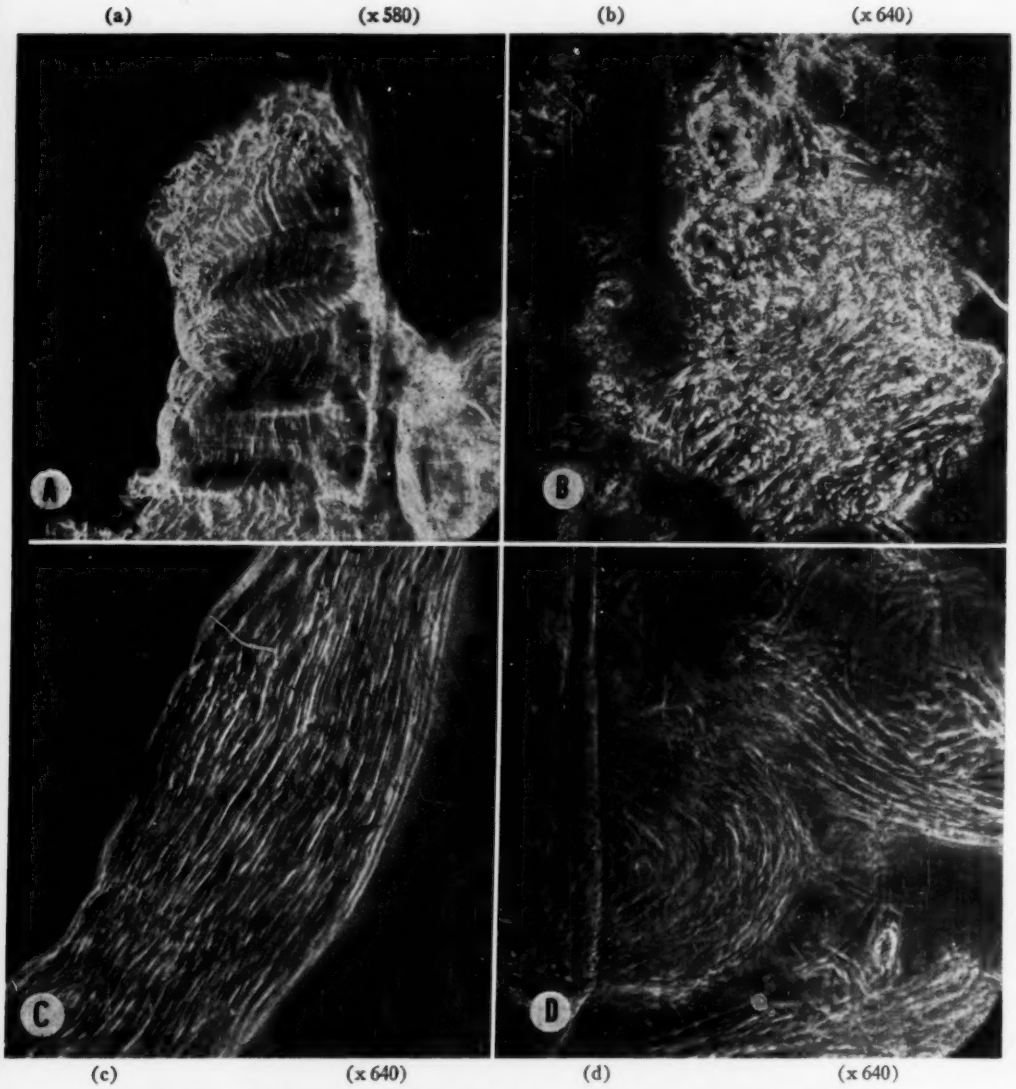


Figure 15. Some interfibrillar splitting phase contrast appearances; autolytic process (a, b) after 35 days/38° C.; epinephrine effect (c, d) after 20 hr./25° C.

and long continuous fibres with alternate shortening-stretching, appearance of a "primary plexiframe striation," while signs of degeneracy, inside or outside the fibre, do not appear.

C. Organoleptic modifications

The organoleptic modifications after epinephrine administration have been observed on cooked and uncooked meat stored for short and long periods under slaughter-house conditions (at 0–5° C.), at normal, and at tropical temperatures (38° C.).

Our observations are mainly related to the external aspect, the colouration, the smell, the flavour, the eating quality, and particularly the tenderness of hog and rabbit muscle. Some data concerning other animals (sheep and ox) are recorded.

1. *Short storage.*

Short storage tests have been carried out. For rabbit, these tests have been conducted for 20 hrs. at 25° C., whereas, for hog and ox, the tests lasted for 72 hrs. at 0 to 5° C.

(a) *Uncooked meat.* All the tested control crude meats lose their elasticity and seem to be shortened and narrowed, whereas the crude meat from adrenalized animals appear unshortened, and extensible.

The original smell remains identical for both the adrenalized and non-adrenalized sample.

The colour seems to have improved. Rabbit and hog are redder, hog more so than rabbit, and beef is darker (Figure 16 see color insert following page 480).

As regards surface moistness and "drip," crude meat from adrenalized animals looks undoubtedly dryer than the control meat.

In Table 3, the organoleptic features of rabbit and hog muscles stored as cited above are compared with some of their metabolic and structural aspects. According to Table 3, improvement of the organoleptic characteristics in the adrenalized samples seems clearly related, with the structural and metabolic modifications.

(b) *Cooked meat.* Grilled pork cutlets, cooked rabbits, and roasted and grilled beef have been tasted as regards their flavour and tenderness.

(i) *Grilled hog cutlets.* Taking into account the possible interanimal variability, the experiment was carried out on 12 hogs. These animals, divided into two groups of 6, controls and adrenalized, were electroshocked, slaughtered, dipped in a water bath at 62° C. for 3 minutes, and stored under normal slaughterhouse conditions, i.e. 72 hrs. at 25° C. prior to cooking. Twenty persons were asked to taste the meat; each person received 4 cooked cutlets, 2 adrenalized and 2 controls, chosen at random, and they did not know their true origin. Unanimously the "adrenalized cooked cutlets" were declared more tender than the controls. We infer statistically that a large population—79.4% (margin of error, 1%) or 86% (margin of error, 5%)—would have found the meat of the 6 adrenalized hogs more tender.

A second experiment has shown that the meat of non-electroshocked adrenalized hogs was also more tender than the meat of the non-electroshocked and non-adrenalized controls. We suggest that the electroshock could strengthen the tenderness but it is not the real determining cause.

TABLE 3

Synopsis of organoleptic, metabolic and structural aspects for hog and rabbit meat (short storage)

		Non-adrenalized (controls)		250 γ/kg. adrenalized	
		Rabbit (20 hr./25° C.)	Hog (72 hr./0–5° C.)	Rabbit (20 hr./25° C.)	Hog (72 hr./0–5° C.)
Organoleptic	Colour	fading	fading	redder	redder
	Moistness (drip)	moist surface	moist surface	dryer surface	dryer surface
	Odour	normal	normal	normal	normal
	Taste*	good taste	good taste	appetizing	appetizing
	Tenderness	tough	very tough	better tenderness	much more tender
Metabolic	pH	5.6	5.8	6.8	6.7
	Exudation	slight (+)	strong (+++) whitish exudate	practically none	slight (+) reddish exudate
Structural	Long intact fibres	45%	15%	75%	55%
	Flexured fibres	predominant	predominant	few	few
	Stretching shortening	few	few	frequent	frequent and strong
	"Plexiframe" striat.	10%	20%	70%	60%
	Interfibrillar-splitting	0	slight (+)	slight (+)	strong (+++)

* After cooking.

Remarks: We have had the opportunity of investigating two very old sows. In tasting their cooked cutlets we found that the dose of 250 γ/kg. induces a slight tenderization. Further experiments are needed for more reliable conclusions.

(ii) *Cooked rabbit meat.* We have tested the organoleptic characteristics of adrenalized and non-adrenalized rabbit meat. A series of animals were slaughtered in the way commonly used for hogs (electroshock, cutting of the throat, immersion at 62° C./3 min.), and another series with the technique normally used for the rabbit.

Twelve persons were asked to eat a sample of the 4 types of cooked rabbit meat: epinephrine, epinephrine-electroshock, control, and control-electroshock; none knew the real origin of each sample. Statistical considerations were as follows: the "non-adrenalized, non-electroshocked" rabbit meat is the hardest. In a large population, at least 68% (margin of error, 1%) or at least 78% (margin of error, 5%) would have found this meat hard.

The "adrenalized, non-electroshocked" samples would be preferred by at least 53% of a large population (tolerated margin of error, 5%).

The "adrenalized, electroshocked" sample seems more tender than the "adrenalized, non-electroshocked," but additional investigation is required.

On the whole, we may conclude that epinephrine induces tenderness in rabbit as well as in hog stored for a short period. As for the enhancing action of the electroshock, the data are not as yet sufficiently conclusive and need to be tested in further work.

(iii) *Cooked beef meat.* Fried steaks, from adrenalized yearling heifers, have been tasted by three consumers.[1] Though being still rather tough, these steaks were considerably more tender than those from control animals. Both controls and "adrenalized" had a very good flavour.

2. *Long storage.*

In our experiments, storage has more or less been prolonged according to the kind of animal and the temperature conditions.

(a) *Freezing storage.* Pork and beef have been stored at 0-5° C., the former for 21 days, and the latter for 31 days.

(i) *Uncooked meat.* Crude meat from adrenalized pigs and heifers presents very satisfactory organoleptic characteristics—in fact, better than those of meat from control animals. Better colour, fresher external and inner aspects, slight surface moistness, very moderate drip, are clearly visible. Moreover, after such a long freezing storage, crude meat looks better than meat from freshly killed adrenalized animals.

(ii) *Cooked meat.* Grilled pork cutlets and fried beef steaks cut from adrenalized animals tasted very tender and had a good flavour, whereas cooked meat from "controls" were tough. Even an adrenalized beef shoulder, cooked as boned rolled roast, tasted tender and had a good, though slight flavour. Statistical evaluations were not made but the results indicated above are the opinions given by several consumers.

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[1] Results recorded through courtesy of Dr. W. D. Bellamy (Research Laboratory, General Electric Co., Schenectady, U.S.A.).

(b) *Tropical temperature storage.* Rabbit, beef and pork have been stored at 38° C. (the former for 14, the latter for 31 and 35 days) and periodically observed.

(i) *Uncooked meat.* All the adrenalized meats kept their own full colour and turgescency while the "controls" grew flaccid, slightly slimy (beef) and pale. On Figure 17 such a difference is clearly visible.

The tenderization seems less pronounced for rabbit than for hog. By investigating with various doses of epinephrine (100 γ/kg. to 1,000 γ/kg.), we found that the tenderness varied from the "firm" to the "flaccid" state through all the intermediate states.

Drip from "adrenalized" meat is but moderate and coloured, whereas on the contrary, drip from "controls" is pronounced and whitish. Both meats from adrenalized or non-adrenalized animals have practically no odour. However owing to storage conditions, they have the similar "tinned tunny" scent.

(ii) *Cooked meat.* Only the pleasant-looking meat from adrenalized animals was tasted. After cooking, their taste was good, even if less tender than fresh "adrenalized" meat.

Miscellaneous data can be reported concerning tests carried out on lamb and horse. The same epinephrine effect on the main organoleptic characteristics: smell, colouration, taste, etc., are found again. Tenderness seems generally enhanced. Investigations in this field are progressing and will be reported elsewhere.

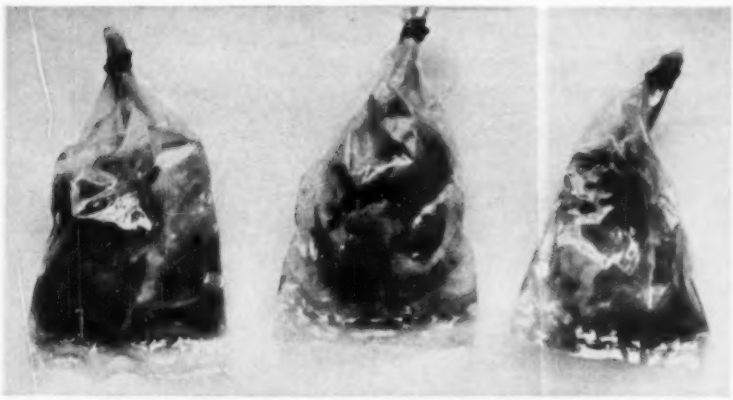
Generally speaking, and if we except a very few minor variations, we may say that the phenomena involved in all skeletal muscle we have tested under ante-mortem epinephrine administration are the same; improvement of the organoleptic characteristics, particularly tenderization, and preservation of the meat by prevention of both the first or exudation phase and the second or metabolic-structural disintegrative phase.

An extra amount of a 250 γ/kg. dose of epinephrine introduced *in vivo* in the organism which normally elaborates and quickly destroys it *(7)* cannot be considered as an "additive" process.

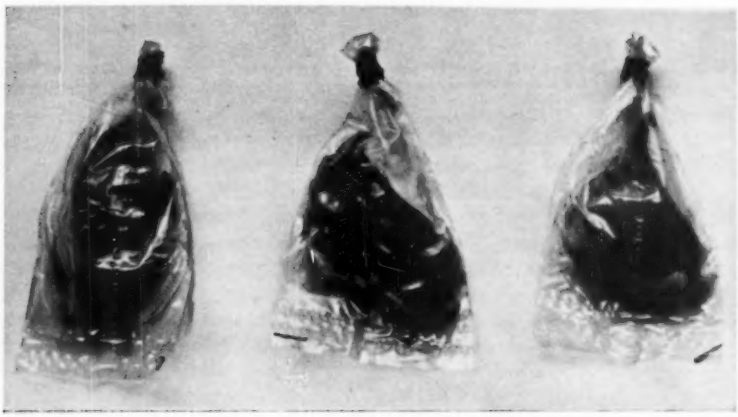
The above results obtained under our experimental aseptic conditions of storage are but an approach to the fundamental problem.

Practical use of the data obtained seems already possible. Healthy meat, when hygienically cut up, is generally sterile *(6b)*. Yet, it can be easily contaminated by saprophytic germs, especially when the pH approaches the physiological pH condition.

Microbial contamination must be forestalled. It is suggested that the anti-autolytic effect of ante-mortem epinephrine administration might well be enhanced by (i) adequate antimicrobial techniques (i.e. surface irradiation *(6)*, static effect of antibiotics, cold and heat sterilization, etc. which can act deeply into the meat), and (ii) by suitable protective packaging. Use of epinephrine as anti-autolytic closely combined with ionizing irradiation methods is recorded in detail elsewhere *(6b)*. Since it assures better meat which remains practically unchanged, even under tropical conditions, this preservation process, based on ante-mortem epinephrine action, can lead to a more efficient distribution of meat and meat products.

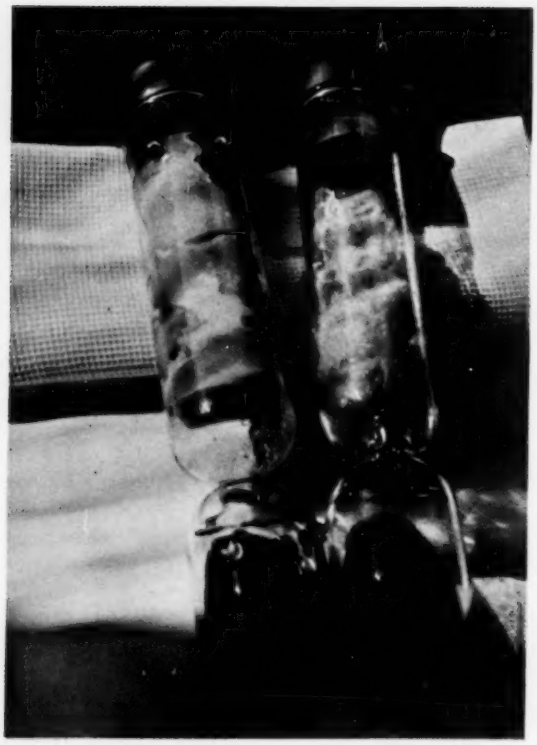


Controls



Epinephrine 250 γ/kg.

Figure 16. Epinephrine effect on organolepty of hog meat (72 hr./0–5° C.).



Adrenalized
Firmness

Controls
Flaccidity
Exudation

Figure 17. Epinephrine effect on organolepty of rabbit meat (14 days/38° C.).

SUMMARY

1. The present work shows the strong preservation effect of ante-mortem epinephrine administration against post-mortem autolysis of skeletal muscle stored under aseptic and anaerobic conditions.

2. Muscles *(Longissimus dorsi)* of laboratory and slaughter-house animals have been investigated (rat, rabbit, lamb, pig, heifer) immediately post-mortem and up to several weeks storage, at freezing (4° C.), normal (25° C.) and tropical (38° C.) temperatures.

3. The *anti-autolytic effect of epinephrine* is established through close investigation of its action on muscle metabolism, structural integrity and organoleptic characteristics.

4. Time/effect and dose/effect relationships have been systematically studied in experiments involving graded doses of epinephrine (10 γ to 1000 γ/kg.) administered at various times prior to killing (0 to 20 hours).

5. A comparative study of non-adrenalized and adrenalized (250 γ/kg. s. c. 4 hours before killing) skeletal muscle shows:

(a) *in the controls,* the normal post-mortem mechanism appearing through:

(i) a first phase of predominantly exudative phenomena characterized by a drop of the pH (towards 5.5), glycogenolysis, high lactic acid level, much sarcoplasmic and myofibrils exudate (high protein level and high $K\cdot/N\cdot$), a rigor contrast phase appearance, and toughness.

(ii) a second storage phase of predominantly autolytic disintegration phenomena, characterized by metabolic, structural, and organoleptic modifications, leading to gradual disruption of the myofibrillar framework and to organoleptic spoiling.

(b) in the *adrenalized samples,* the inhibition of the aseptic autolytic process setting in through:

(i) a first phase of "glucidic-lactic acid" level depression, pH drop inhibition and exudation prevention, fresh muscle phase contrast appearance (alternate stretching-shortening band pattern).

The various organoleptic improvements (external aspect, colour, flavour, mouth feel) have been observed.

Concerning the palatibility, an obvious improvement of the tenderness of cooked meat has been statistically evaluated.

(ii) a second storage phase in which the metabolic and structural disintegration modulus appears to be stopped or strongly reduced: glycogenolysis, physiological pH, turgescency and structural integrity at fibre and myofibril levels, improvement of organoleptic characteristics which will keep even at tropical temperatures; amino acid genesis is reduced, and glycine-soluble-protein electrophoretic diagrams do not show characteristic new protein sub-unit peaks comparable to those of the "controls."

6. The use of epinephrine as a new anti-autolytic non-additive process is discussed.

7. Being harmless and producing a better meat resistant to long storage, even in tropical countries, the process described allows a more efficient meat distribution which can be even further improved if associated with some adequate physicochemical antimicrobial techniques.

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ORGANIC ACIDS IN COFFEE IN RELATION TO THE DEGREE OF ROAST

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The chemistry of flavor development during the roasting of coffee is not well understood. Acidity is often considered an important factor in the flavor of brewed coffee. Consequently, a study of the acidic compounds of coffee might contribute to a better understanding of the taste and flavor of coffee beverages. Kaufman *(8)* made water extracts of both green coffee and successive samples withdrawn during roasting, and observed that the pH fell about one pH unit, and then began to rise. These changes were confirmed here. The optimum roast is often considered to terminate shortly after the pH of the water extract begins to increase. The titratable acidity is nearly doubled at this point *(12, 16)*. Kaufman *(8)* suggested that these changes were due to the formation and volatilization of acetic acid from decomposition of sugars, decarboxylation of acids formed by rearrangement of sugars and decarboxylation of chlorogenic acid. In a heavy roast approximately 50% of the original chlorogenic acid was reported to be destroyed *(14)*. Since partition chromatography on silica gel was particularly adaptable for a separation of organic acids in coffee *(9, 11, 19)* a quantitative study of several organic acids in coffee during roasting has been carried out.

EXPERIMENTAL

Materials. Coffee samples were obtained from the Coffee Brewing Institute, New York. The samples were vacuum packed in one-pound cans as whole beans and stored at −20° C. Table 1 describes the samples and gives the code.

Methods. The weight of 100 beans was obtained as a mean value, weighing 4 groups of 200 beans each. One hundred grams of coffee beans were ground coarsely with a laboratory mill. Moisture was determined by drying 2 g. for 5 hours at 105°–110° C. Thirty-gram samples were shaken 5 minutes with 400 ml. distilled water of 95° C. in a 1000-ml. Erlenmeyer flask. Next, 100 ml. of distilled water was added by rinsing the walls of the flask; the flask was then cooled under running tap water. The extract was centrifuged for 20 minutes and the solution transferred to a 500-ml. volumetric flask and filled to the mark. Using 25 ml. of this extract the titratable acidity was detrmined by titrating with 0.1 N sodium hydroxide to pH 8.2 as indicated by a Beckman pH meter model G. The remaining extract was brought to pH 7.9 with 0.1 sodium hydroxide and after filling again to the mark 20 ml. aliquots were freeze-dried. The weight of solids was recorded and then the solid extract after acidifying to pH 1 was transferred to the top of a column of silica gel. The organic acids were then separated into 6 bands by a method similar to that reported from this laboratory *(11)*. The purity of these bands was checked by paper chromatographic methods using the R_f values of Table 2 in the case of phenolic acids. Some of the compounds were identified by physico-chemical methods.

The individual fractions from the column were evaporated to dryness. If the presence of volatile acids was suspected, the fractions were neutralized with ammonia before drying. The residues were dissolved in ethanol or water and the solutions were applied in form of spots on Whatman number 1 filter paper along with spots of known compounds.

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Chlorogenic and caffeic acids were obtained commercially. Isochlorogenic acid was isolated by the method of Barnes *et al. (2)* using isobutyl acetate rather than butyl acetate. The crude acid was purified by chromatography on silica gel. The papers were then developed in the ascending manner with the appropriate solvents and the acids revealed by the appropriate reagents. The solvents were of analytical grade if so obtainable. Chromatography was conducted at room temperature.

TABLE 1

Sampling temperature

Type coffee	Green	300° F.	325° F.	350° F.	375° F.	400° F.	Final roast	Final temp.
A. Santos	1A	2A	3A	4A	5A	....[1]	7A	402° F.
B. Maracaibo-Cuban	1B	2B	3B	4B	5B	6B	7B	419° F.
C. Medellin-Armenia	1C	2C	3C	4C	5C	6C	7C	421° F.
D. Post Crop Colombia	1D	2D	3D	4D	5D	6D	7D	430° F.
E. Old Crop Bucara.	1E	2E	3E	4E	5E	6E	7E	431° F.
F. Peruvian	1F	2F	3F	4F	5F	6F	7F	413° F.
G. Central America	1G	2G	3G	4G	5G	6G	7G	407° F.

[1] Insufficient time remained for a sampling before completion of roast.

Band 1: Band 1 was eluted from the column with 45 ml. 5% n-butanol-chloroform. Earlier work in this laboratory showed the presence of propionic, butyric, and valeric acids in this band *(5)*. The first 25 ml. of the eluate contained caffeine, which was identified by paper chromatography with 2% acetic acid as solvent. The spot was located by ultraviolet light and after elution the ultraviolet absorption spectrum was compared to known caffeine.

TABLE 2

Paper chromatography of phenolic acids of coffee

Material	R_f				Indicator	
	1	2	3	4	Ultraviolet light	Ultraviolet light and ammonia
Caffeic acid	0.26 (0.58)	0.91	0.13	0.20	blue	blue
Acid A	0.58 (0.71)	0.82	0.13	0.22	blue	blue-yellow
Acid B	0.56 (0.71)	0.80	0.07	0.23	blue	blue-yellow
Acid C	0.63	0.79	0.03	0.30	.....	blue
Isochlorogenic acid	0.11 (0.22)	0.90	0.00	0.18	blue	yellow-green
Chlorogenic acid	0.53 (0.70)	0.71	0.00	0.18	blue	yellow-green
Acid D[1]	0.49 (0.69)	0.69	0.00		blue	yellow-green
Neochlorogenic acid	0.59 (0.71)	0.66	0.00		blue	yellow-green

Solvents: 1. 2% acetic acid, two spots indicate probably the presence of cis-trans isomers *(23)*. 2. Ethyl acetate–acetic acid–water 2-1-1. 3. Chloroform–acetic acid–water 2-1-1. 4. Isopropyl alcohol–ammonia–water 8-1-1, caffeic acid and its esters gave streaks, probably because of oxidation and polymerisation.

[1] Also reported as compound or band 510 *(9, 19)*.

Band 2: Band 2 was eluted with an additional 55 ml. 5% n-butanol-chloroform. Acetic acid was identified by paper chromatography by the method of Reid and Lederer *(17)*; caffeic acid and a further phenolic acid A were separated by paper chromatography. Acid A appears to be a quinic acid ester of ferulic acid *(9)*.

Band 3: Band 3 was next eluted with 65 ml. 15% n-butanol-chloroform. Isochlorogenic acid and the phenolic acids A, B and C were separated by paper chromatography. Acid B was earlier tentatively identified as a second quinic acid ester of ferulic acid and acid C as a quinic acid ester of p-coumaric acid *(9)*. Separation of formic acid from the phenolic acids in this band was achieved by chromatography on a second column in the way described by Bulen *et al. (3)* for the separation of glutaric, fumaric, and formic acids. Formic acid was further identified by paper chromatography by the method of Reid and Lederer *(17)*.

Band 4: Band 4 was eluted with 75 ml. 15% n-butanol-chloroform and 100 ml. 25% n-butanol-chloroform. Chlorogenic acid, the predominant acid in this band, and acid D, which seems to be another chlorogenic acid isomer *(9)*, were separated by paper chromatography. If the amount is low, chlorogenic acid may be separated from acid D by elution with the first solvent.

Band 5: Band 5 was eluted with 110 ml. 35% n-butanol-chloroform. Neochlorogenic acid was found by paper chromatography and malic acid, the predominant acid in this band, was isolated from the last 70 ml. of the eluate and identified by its melting point.

Band 6: Band 6 was eluted with 190 ml. 35% n-butanol-chloroform. Citric acid was isolated from the eluate and identified by its melting point.

In bands 1, 2 and 3 perhaps other phenolic compounds and keto acids were present. The presence of several keto acids in green and roasted coffee was reported earlier from this laboratory *(5)*. Oxalic acid was not detected in band 4. No tartaric acid was detected in a further band, which was eluted from the column with 50% n-butanol-chloroform; but quinic acid was found in this band and identified by paper chromatography by the method of Cartwright and Roberts *(4)*. The high and somewhat variable blank value of this eluate made an accurate determination of a small amount of quinic acid more difficult.

Isolated isochlorogenic acid and coffee extracts were checked for the presence of the lactone of isochlorogenic acid because Barnes *et al. (2)* postulated an equilibrium between the free acid and its lactone. Chromatography on paper and silica gel gave no evidence for the presence of the lactone.

RESULTS AND DISCUSSION

A hot water extract resembles very closely the form in which coffee is used as a beverage; furthermore, a hot water extract has been proposed for the analysis of chlorogenic acid in coffee *(13)*. The portion of a particular organic acid in the extract depends on its solubility in water. The values reported are only related to the water extract under defined conditions. In case of coffee 1C the ratio of chlorogenic acid to isochlorogenic acid in the water extract was 10:1 if band 4 from Table 3 was calculated as chlorogenic acid and band 3 as isochlorogenic acid. If the extraction was repeated with the residue the ratio of these acids was 6:1. Earlier work in this laboratory *(11)* has shown that 75%–88% of the estimated acids were extracted in the first brew.

Although it was proposed to grind coffee at least 30 mesh for a determination of chlorogenic acid *(21)*, no sieving of the ground coffee was carried out. A preliminary study of the particle size distribution did not show great differences in the case of coarsely ground coffee of various degree of roast, whereas green coffee ground under the same conditions consisted of larger particles than roasted coffee. Prolonged grinding of green coffee may destroy some of the heat labile compounds of coffee. Weiss *(20)* reported that the effect of particle size on the efficiency of extraction of chlorogenic acid is obscured because of apparent destruction of this acid during grinding. The extract was separated from the residue by centrifuging although it was not possible, especially in the case of green coffee, to get a clear solution, probably because of the presence of fat, oil and various colloidal materials.

Organic acids found. Part of the results of this study are to be seen in Table 3. For the sake of brevity the data on the Central American samples (G) were deleted since they are similar to the results on the Peruvian samples (F); also the Maracaibo-Cuban (B) and Old Crop Bucaramanga (E) are not given as they were similar to findings on the Post Crop

TABLE 3

The acidity of coffee in relation to the degree of roast

Sample	Weight of 100 beans	Moisture in %	Ml. extract	Neutralized extractable matter in %	pH	Acids, as ml. 0.1 N NaOH per 1 g. fresh weight						
						Titratable	Band					
							1	2	3	4	5	6
1A	11.8	4.72	430	29.0	5.88	1.20	0.03	0.11	0.18	1.39	0.74	1.68
3A	10.7	2.60	441	26.6	5.78	1.27	0.03	0.17	0.21	1.40	0.82	1.57
4A	10.7	1.70	446	26.2	5.48	1.45	0.06	0.23	0.27	1.23	0.81	1.62
5A	10.6	1.65	440	25.5	5.18	1.85	0.09	0.40	0.46	1.24	0.83	1.39
7A	10.7	2.20	446	25.7	5.12	1.88	0.12	0.51	0.56	1.00	0.71	1.21
1C	17.5	6.16	428	29.4	5.98	1.15	0.01	0.11	0.14	1.42	0.60	1.54
2C	16.0	4.50	435	26.5	5.85	1.19	0.01	0.18	0.15	1.52	0.67	1.66
3C	15.7	4.00	435	25.2	5.68	1.27	0.03	0.19	0.20	1.44	0.71	1.62
4C	15.6	2.30	437	26.4	5.31	1.52	0.09	0.33	0.33	1.33	0.75	1.61
5C	15.4	1.58	447	26.8	5.01	2.26	0.10	0.50	0.51	1.30	0.75	1.27
6C	14.6	1.38	445	27.8	5.10	2.05	0.10	0.61	0.52	1.02	0.64	0.99
7C	14.5	1.68	440	28.0	5.20	1.91	0.12	0.64	0.55	0.89	0.57	0.90
1D	15.9	4.80	444	28.0	5.83	1.31	0.04	0.10	0.24	1.33	0.88	1.45
3D	14.2	2.60	443	24.4	5.73	1.33	0.04	0.14	0.23	1.46	0.84	1.46
4D	14.0	2.17	446	24.4	5.41	1.53	0.04	0.20	0.33	1.15	0.86	1.27
5D	13.8	1.35	441	25.2	5.16	1.87	0.08	0.41	0.45	1.13	0.79	1.02
6D	13.7	1.20	442	25.1	5.23	1.67	0.10	0.46	0.53	1.00	0.68	0.92
7D	13.5	0.90	437	26.2	5.48	1.42	0.10	0.47	0.48	0.90	0.63	0.81
1F	18.3	4.45	434	28.2	5.95	1.20	0.03	0.09	0.16	1.44	0.61	1.68
3F	16.7	3.89	435	27.7	5.70	1.33	0.04	0.13	0.22	1.32	0.75	1.59
4F	16.3	3.25	446	28.2	5.38	1.54	0.05	0.24	0.30	1.26	0.71	1.48
5F	15.6	2.08	446	26.8	4.98	2.00	0.08	0.46	0.53	1.09	0.69	1.21
6F	15.4	1.12	441	26.6	5.08	1.94	0.09	0.51	0.57	0.91	0.66	0.97
7F	15.0	1.75	443	26.8	5.20	1.82	0.12	0.53	0.62	0.96	0.55	0.82

Colombian results (D). The data are related to the extract of 1 g. fresh coffee, but they are distorted because of the loss in weight during the roasting of coffee beans. The weight of 100 beans was determined in order to follow this loss in weight. Relating the data to the number of beans rather than to fresh weight or dry substance seems to be advantageous providing the coffee is stored under known and constant conditions. In Figures 1, 2, 3 and 4 data from Table 3 were related to the number of beans. A comparison of green and roasted coffee on the basis of dry weight is questionable because the loss in weight during roasting far exceeds the loss of water. Only two of the second series of coffee were analysed. In this early stage of roasting no characteristic changes in the organic acids were detectable. Coffee A had a very uneven distribution of bean size. This fact was apparently the reason for the irregularity in the weight of 100 beans of this coffee during roasting.

With the exception of formic acid, which seems to be a decomposition product rather than native to coffee, all identified acids—butyric, valeric and propionic *(5)*, acetic, malic, citric, quinic, caffeic, chlorogenic, isochlorogenic and neochlorogenic acids as well as the phenolic acids A, B, C and D were present in green as well as in roasted offee. Caffeic acid was hardly detectable in extracts of green coffee. The main part of the ultraviolet absorption of band 2 was due to acid A. There was more caffeic acid present in roasted coffee, but a semi-quantitative analysis by eluting this acid from a paper chromatogram and estimating the absorption at 320 mμ showed that the amount of caffeic acid in roasted coffee was below 0.05%. Caffeic acid because of its ease of decarboxylation is not considered to be a decomposition product of the chlorogenic acids. The very unstable p-vinyl catechol, found in extracts of roasted coffee *(15)*, seems to be formed by decomposition of the chlorogenic acids. It seems very probable that p-vinyl guaiacol, which was isolated from coffee extracts *(15)*, is a decomposition product of the esters of ferulic acid. Quinic acid, which was only present in traces in the extract of green coffee, increased during the roast; however it was only a small fraction of the amount of quinic acid calculated from the decomposition of chlorogenic acid. The quinic acid part of the chlorogenic acids seems to decompose further or may form the lactone. No oxalic acid was detected and from this fact it can be assumed that oxalic acid, which was reported by several authors to be present in coffee *(1, 7, 22)* may be present in form of the water insoluble calcium salt rather than as the free acid.

Changes in total soluble matter, acidity, and pH during roasting. The percentage of the water soluble material in coffee decreased after starting the roast but remained more or less constant in the later stages of roasting. The changes of the acidity and pH of extracts of coffee of different degree of roast were similar to those observed by other authors *(8, 12, 16)*. With exception of the Santos (A) and Central America (G) coffees samples at number 5 roast had the lowest pH. Coffee A and G consisted of small beans and the final roasting temperature was lower than with the other coffee; here the pH between roasts number 5 and number 7 showed only little change.

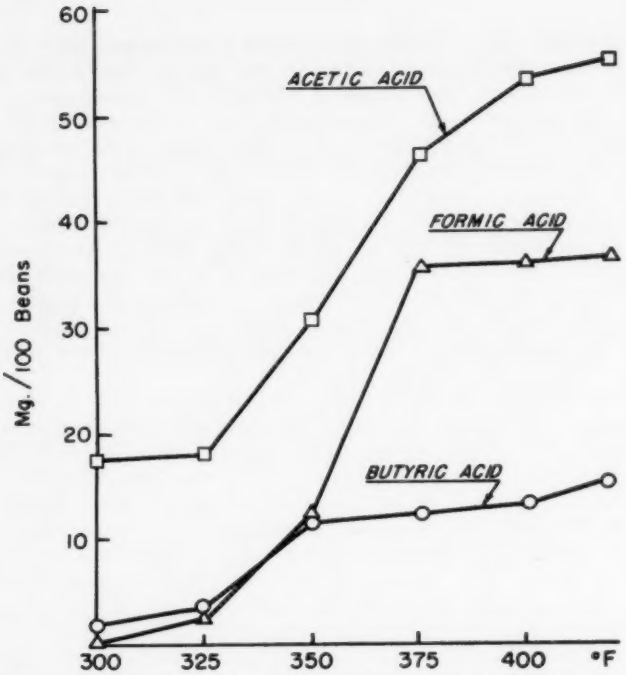

Figure 1. Formation of acids during roasting of coffee (Medellin-Armenia).

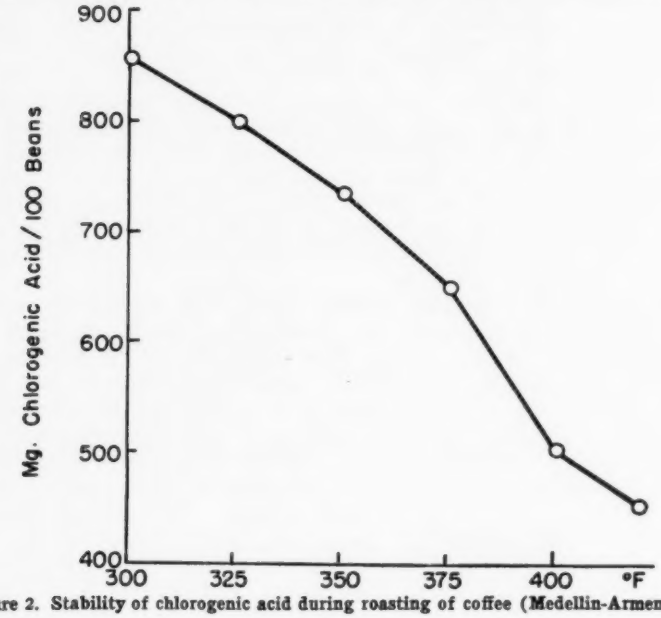

Figure 2. Stability of chlorogenic acid during roasting of coffee (Medellin-Armenia).

Decomposition and formation of acids during roasting. In the earlier stages of roast the decomposition of the phenolic acids—especially chlorogenic acid in band 4—citric and malic acid was more than compensated by the formation of volatile acids, especially formic and acetic acids, and hence the pH of the extracts decreased. In roasting the coffee further the the amount of volatile acids remained relatively constant due to formation and volatilisation at the same time, whereas the non-volatile acids decomposed further and the pH rose. Figures 1, 2, 3 and 4 show the changes of some acids of coffee C calculated as mg. in 100 beans. In Figure 1 band 1 was calculated as butyric acid, band 2 as acetic acid, and band 3 as formic acid. In the case of formic acid, correction was made for the presence of phenolic acids in this band. In Figure 2 band 4 was calculated as chlorogenic acid, in Figure 3 band 5 was calculated as malic acid and in Figure 4 band 6 was calculated as citric acid. Malic acid seems to be more stable than chlorogenic and citric acids. The decomposition of malic acid started at 350° F., whereas citric and chlorogenic acids decomposed at 300° F. Calculating band 4 as chlorogenic acid, band 5 as malic acid and band 6 as citric acid the decomposition at the final roast (related to 100 beans) of malic acid was in the range of 16% (coffee A) to 40% (coffee E), of chlorogenic acid in the range of 32% (coffee G) to 51% (coffee E) and of citric acid in the range of 33% (coffee A) to 56% (coffee D). In coffee with a higher final roast temperature the decomposition was greater than in coffee with a lower final roast temperature.

Only approximately one third of the acids found by chromatography in green coffee was titratable in the water extract. From this analysis no decision is possible as to which acids or fractions of the acids were present free or which were present as salts. It is reported *(10)* that chlorogenic acid is present in coffee partly as free acid, partly as potassium salt and partly as double salt between the potassium salt and caffeine. According to Griebel *(6)* chlorogenic acid is present only as salt, probably as calcium, magnesium and potassium salts. The decomposition starts with the double salt according to Lindner *(10)*, but according to other authors with the potassium salt, the double salt being more heat resistant *(18)*. It can be only suggested from this study that in the beginning of the roast the salts of chlorogenic and citric acids decomposed rather than the free acids, as indicated by the parallel increase in the formation of formic and acetic acids and titratable acidity. In the latter part of roasting the titratable acidity decreased simultaneously with the decomposition of chlorogenic, malic and citric acids, whereas the amounts of acetic and formic acids were more or less constant. From this fact it can be concluded that in this stage free chlorogenic, malic and/or citric acids decomposed.

The hitherto unsuspected complexity of the phenolic acids of coffee requires a critical interpretation of results of determinations of chlorogenic acid. Present analytical methods for chlorogenic acid determine either the whole complex of phenolic acids or differentiate only between more water soluble and more ether or ethyl acetate soluble acids. At the present time column chromatography on silica gel in connection with measuring the absorbance of the eluate in the ultraviolet range *(19)* seems to be advantageous for a separation and determination of phenolic acids, including

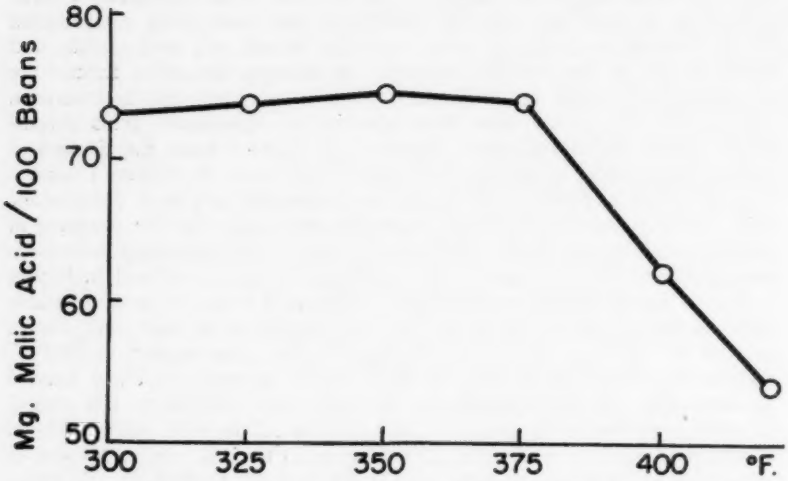

Figure 3. Stability of malic acid during roasting of coffee (Medellin-Armenia).

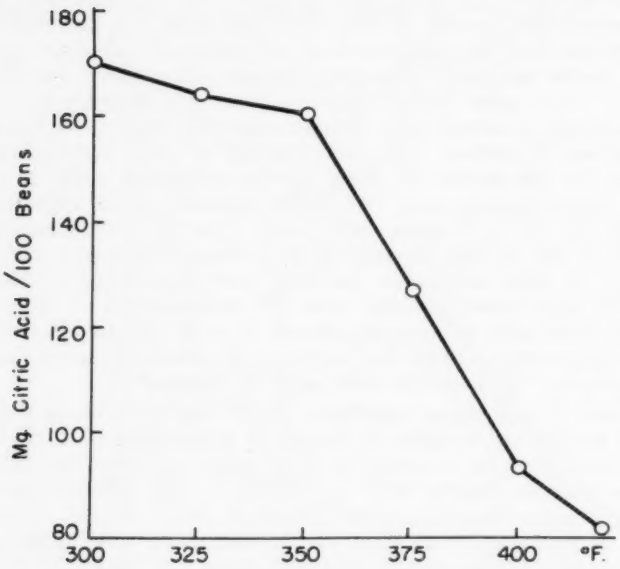

Figure 4. Stability of citric acid during roasting of coffee (Medellin-Armenia).

chlorogenic acid. In the case of roasted coffee the colored decomposition products interfere with the spectrophotometric determination of the phenolic acids and a prior separation of these products and the phenolic acids is necessary.

SUMMARY

The organic acids in hot water extracts of various coffee in relation to the degree of roast were studied by a combination of partition chromatography on silica gel and paper chromatography. R_f values of several phenolic acids are given. The following acids were found to be present in green as well as in roasted coffee: Acetic, propionic, butyric, valeric, malic, citric, quinic, caffeic, chlorogenic, isochlorogenic, neochlorogenic, a further isomer of chlorogenic, p-coumaryl quinic and two isomers of ferulyl quinic. Formic acid was found only in roasted coffee. The predominant acids in coffee were chlorogenic and citric. Caffeic and quinic acids were present only in traces. During roasting the amounts of formic and acetic acids increased. Between 32% and 52% of the chlorogenic acid was destroyed during roasting, whereas the loss in citric acid was 33% to 56% and for malic acid the loss was 16% to 40%, depending on the particular coffee and the final roast temperature. This simultaneous formation and decomposition of organic acids during roasting of coffee explains why the pH of coffee brews reaches a minimum with a light roasted coffee.

Acknowledgment

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COMPOSITION OF CERTAIN SPECIES OF FRESH-WATER FISH. II. COMPARATIVE DATA FOR 21 SPECIES OF LAKE AND RIVER FISH

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Part 1 of this series of papers *(2)* presented information on factors possibly bearing upon the variation in composition of fish and described the need for such data. Twenty-one species of fresh-water fish now have been investigated—some of them in samples of sufficient range and size to indicate that certain factors do have a definite influence on composition. In the present paper, however, primary attention is focussed on the ranges and averages of the nutritional components of fresh-water fish classified according to species. Only minor consideration is given to the influence of other factors at this time because they will be given detailed consideration in a later report.

EXPERIMENTAL

Source of specimens. The fish reported in this paper represent 21 species and several sub-species that were collected in the states of Iowa, Michigan, Minnesota, Montana, Oregon, Ohio, Washington, and Wisconsin (Table 1). They were taken from 4 of the Great Lakes: Erie, Huron, Michigan, and Superior; 7 lesser lakes: Benton, Ripley, Shetek, Red Lake, and Two Rivers Lake in Minnesota, Lake Hibgen in Montana, Lake Winnebago in Wisconsin, and from 2 rivers, the Columbia and the Mississippi.

Collection of specimens. The fish were collected over a period of 7 years; however, in only one species, sheepshead, were sufficient samples obtained during different seasons to allow comparison of seasonal differences. They will be reported in greater detail in a later paper when this part of the over-all project has been completed.

From the point of collection, the fish were sent to Seattle either by rail or by air express. The usual procedure was to pack the fish in ice as soon as they were caught and to ship them, within 24 hours, by rail. Time in transit ranged from 2 to 4 days, and the fish, upon arrival, were found to be of uniformly good quality. After being received at the Seattle laboratory, they were packed in plastic bags, frozen, and stored at −18° C. until samples were prepared for analysis. The fish that were sent by air express were packed in plastic bags at the point of collection and then were frozen. On arrival at Seattle, they also were stored at −18° C. until prepared for analysis.

Preparation of samples. In the preparation of samples for analysis, the fish were thawed in lukewarm water, washed, measured, and weighed. Fillets were removed from both sides of the fish and were skinned. Skinned fillets were weighed and then were ground in a food chopper. The ground flesh was packed in ½-pound cans, vacuum-sealed, frozen, and stored at −18° C. until the analyses were made.

For some samples, the fillets from the individual fish were kept separate. For others, the fillets of all of the fish in the given lot were combined to form a composite sample. A composite sample also was prepared from the carcass, viscera, and skin of the fish in most of the series.

Procedure. The procedure for making an analysis of a can of frozen sample was as follows: (1) Thaw the ground flesh by immersing the can in lukewarm water for

* Now retired.

TABLE 1

Source data for fresh-water fish

Central States Area

Series No.	Common name	Scientific name	Place of capture		Date	
			Body of water	State	Mo.	Year
1	Bass, white	*Lepibema chrysops*	Lake Winnebago	Wis.	7	53
2	Buffalo	*Ictiobus* species	Mississippi River	Iowa	6	52
3	Buffalo	*Ictiobus* species	Mississippi River	Iowa	4	57
4	Bullhead	*Ameiurus melas melas*	Lake Benton	Minn.	8	52
5	Bullhead	*Ameiurus melas melas*	Lake Shetek	Minn.	8	53
6	Burbot	*Lota lota maculosa*	Lake Erie	Ohio	11	55
7	Burbot	*Lota lota maculosa*	Lake Erie	Ohio	11	55
8	Carp	*Cyprinus carpio*	Mississippi River	Iowa	5	52
9	Carp	*Cyprinus carpio*	Lake Benton	Minn.	8	52
10	Carp	*Cyprinus carpio*	Lake Erie	Ohio	11	55
11	Carp	*Cyprinus carpio*	Mississippi River	Iowa	4	57
12	Chub	*Leucichthys* species	Lake Michigan	Mich.-Wis.	7	52
13	Chub	*Leucichthys* species	Lake Michigan	Mich.-Wis.	8	53
14	Chub	*Leucichthys alpenae*	Lake Michigan	Mich.-Wis.	9	54
15	Chub	*Leucichthys alpenae*	Lake Michigan	Mich.-Wis.	9	54
16	Chub	*Leucichthys zenithicus*	Lake Michigan	Mich.-Wis.	9	54
17	Chub	*Leucichthys kiyi*	Lake Michigan	Mich.-Wis.	9	54
18	Chub	*Leucichthys hoyi*	Lake Michigan	Mich.-Wis.	9	54
19	Chub	*Leucichthys reighardi*	Lake Michigan	Mich.-Wis.	9	54
20	Crappie	*Promoxis annularis*	Two Rivers Lake	Minn.	10	53
21	Gizzard shad	*Dorosoma cepedianum*	Lake Erie	Ohio	11	55
22	Herring	*Leucichthys artedi*	Lake Superior	Minn.	12	54
23	Herring	*Leucichthys artedi*	Lake Superior	Minn.	7	55
24	Herring	*Leucichthys artedi*	Lake Huron	Mich.	11	55
25	Herring	*Leucichthys artedi*	Lake Superior	Minn.	11	55
26	Herring	*Leucichthys artedi*	Lake Superior	Minn.	12	55
27	Mullet	*Catostomidae* species	Lake Huron	Mich.	11	55
28	Mullet (Sucker)	*Catostomidae* species	Red Lake	Minn.	7	57
29	Perch, yellow	*Perca flavescens*	Lake Erie	Ohio	10	51
30	Perch, yellow	*Perca flavescens*	Lake Erie	Ohio	6	52
31	Perch, yellow	*Perca flavescens*	Lake Erie	Ohio	4	57
32	Pike, blue	*Stizostedion vitreum glaucum*	Lake Erie	Ohio	10	51
33	Pike, blue	*Stizostedion vitreum glaucum*	Lake Erie	Ohio	6	52
34	Pike, yellow	*Stizostedion vitreum vitreum*	Lake Erie	Ohio	10	55
35	Pike, yellow	*Stizostedion vitreum vitreum*	Lake Erie	Ohio	6	52
36	Pike, yellow	*Stizostedion vitreum vitreum*	Lake Erie	Ohio	4	51
37	Pike, northern	*Esox, lucius*	Lake Ripley	Minn.	2	55
38	Sheepshead (L.)	*Aplodinotus grunniens*	Lake Winnebago	Wis.		(51-7)
39	Sheepshead (R.)	*Aplodinotus grunniens*	Mississippi River	Iowa		(52-7)
40	Smelt, lake	*Osmerus mordax*	Lake Michigan	Mich.	3	52
41	Trout, lake	*Cristivomer namaycush namaycush*	Lake Superior	Minn.	6	52
42	Trout, lake	*Cristivomer namaycush namaycush*	Lake Superior	Minn.	8	54
43	Trout, lake	*Cristivomer namaycush siscowet*	Lake Superior	Minn.	9	54
44	Whitefish	*Coregonus clupeaformis*	Lake Erie	Ohio	11	51
45	Whitefish	*Coregonus clupeaformis*	Red Lake	Minn.	8	54
46	Whitefish	*Coregonus clupeaformis*	Red Lake	Minn.	8	54

Western States Area

47	Smelt, river	*Thaleichthys pacificus*	Columbia River	Ore.	4	52
48	Utah chub	*Gilo atraria*	Lake Hibgen	Mont.	7	53
49	Squawfish	*Ptychocheilus oregonensis*	Columbia River	Wash.	1	53
50	Squawfish	*Ptychocheilus oregonensis*	Columbia River	Wash.	1	53
51	Squawfish	*Ptychocheilus oregonensis*	Columbia River	Wash.	1	53
52	Squawfish	*Ptychocheilus oregonensis*	Columbia River	Wash.	1	53

(L.)—Lake; (R.)—River.

TABLE 2

Physical data for fresh-water fish

Series No.	Quantity	Common name	Length		Weight		Fillet yield	
			Av.	Range	Av.	Range		Range
			cm.	*cm.*	*grams*	*grams*	%	%
1	3	Bass, white	27.7	27 –29	330	150– 465		
2	7	Buffalo	47.8	36 –61	2025	915–3795	33.7	23.6–35.3
3	6	Buffalo			3335			
4	16	Bullhead	26.0	25.0–28.0	284	217– 323	18.9	12.3–24.4
5	40	Bullhead	18.7		104			
6	9	Burbot	44.7	37 –56	790	410–1358		
7	2	Burbot	56.0		1719		15.3	
8	16	Carp	53.9	45 –64	1850	1230–2935	29.6	24.3–35.0
9	16	Carp	43.6	25 –55	1348	265–2080	28.9	25 –32
10	9	Carp	30.0	26 –34	670	381– 952	24.5	22 –27
11	6	Carp	48.2		2332			
12	16	Chub	23.1	20 –33	116	85– 160	*40.7	33.7–48.3
13	16	Chub	25.4	24 –27	134	110– 155		
14	7	Chub	23.7		173		32	
15	13	Chub	23.2		160		33	
16	4	Chub	23.3		153		33	
17	17	Chub	22.4		144		31	
18	16	Chub	18.8		90			
19	16	Chub	22.6		159		35	
20	9	Crappie	21.3	18 –30	182	98– 460		
21	16	Gizzard shad	29.6	27.5–35	498	366– 722	32.8	31.7–37.2
22	16	Herring	25.6	24.5–26.5	167	135– 202		
23	12	Herring						
24	16	Herring	26.3	25 –28	205	168– 270	37.7	31.5–44.7
25	16	Herring	27	24 –29	218	164– 270		
26	16	Herring	26	23 –28	185	149– 250	34.3	29.3–43.3
27	3	Mullet	36	35 –36.5	165	85– 224		
28	11	Mullet (Sucker)	31.7	29.5–37.0	550	400– 856	35.2	32.2–38.1
29	16	Perch, yellow	23.3	20.5–26.5	199	125– 285	39.3	36.0–42.8
30	16	Perch, yellow			174		33.2	
31	6	Perch, yellow	23.6		223			
32	16	Pike, blue	29.1	26.5–32.0	196	145– 280	(1)43.9	41.3–46.9
33	16	Pike, blue			285		34.4	
34	16	Pike, yellow	42.6	41 –58	784	360–2165	(1)57.4	53 –62
35	16	Pike, yellow			400		34.7	
36	6	Pike, yellow	42.5		988			
37	10	Pike, northern	57.1	47.5–66	1586	825–2298	26	22 –29
38	480	Sheepshead (L.)			605	435– 916		
39	155	Sheepshead (R.)			676	347–1191		
40	16	Smelt, lake						
41	12	Trout, lake	68.7	54 –94	2813	930–6804	(1)58.5	
42	3	Trout, lake	63.3	50 –73	2797	1188–3913		
43	15	Trout, lake	53.7		2672	610–9445		
44	16	Whitefish	45.6	38 –55	883	600–2285	*47.0	39 –54
45	3	Whitefish	42	32 –47	1059	502–1396	*47.9	45 –50
46	6	Whitefish						
47	16	Smelt, river						
48	16	Utah chub		24.5–33.5	326			
49	8	Squawfish	36	32 –42	522	405– 800	31.4	28.8–34.5
50	16	Squawfish	24.5		187			
51	9	Squawfish	32.5		353			
52	8	Squawfish	36.7		548			

* Fillet yield calculated with skins but skins removed for analysis.
(1) Yield based on eviscerated fish.

20 to 30 minutes. (2) Open the can and stir the contents thoroughly to make certain that any separated liquid will be homogeneously reincorporated into the sample. (3) Transfer approximately 40 g. of the ground fish to a beaker and homogenize with a high-speed cutting and mixing blade. (4) Transfer the finely ground flesh to a container with a tight cover to prevent loss of moisture. (5) Remove samples of the ground flesh from the covered container and, using the procedures described in *Methods of Analysis* of the Association of Official Agricultural Chemists *(1)*, analyze them in duplicate for the constituents: (a) moisture, (b) oil, (c) protein, and (d) ash, as follows: (a) Determine the amount of moisture by measuring the weight lost by 5-g. samples of the ground flesh dried in a forced-air oven at 101° C. for 4 hours.

(b) Determine the amount of oil by weighing the oil obtained from 3-g. samples of the ground flesh by acid hydrolysis and solvent extraction. (c) Determine the amount of crude protein by assaying 3-g. samples of the ground flesh for nitrogen by the Kjeldahl procedure and by applying the factor of 6.25 for conversion of nitrogen to protein. (d) Determine the amount of ash by weighing the residue from 4-g. samples of ground flesh that have been ignited in a muffle furnace at 550° C.

The samples of fish received since 1956 have been analyzed also for sodium and potassium by the use of a direct-reading flame photometer which became available at that time. The method employed was adapted from one developed by chemists at the research laboratory of the National Canners Association *(4)*. The procedure is as follows: (a) Dissolve the mineral consituents of the ashed sample by treating it with nitric acid. (b) Filter the resultant mixture. (c) Dilute the filtrate to an appropriate concentration for reading in the photometer.

RESULTS AND DISCUSSION

The data can be treated conveniently in 5 sections as follows: (1) variation in physical data, (2) variation in proximate composition of fillets, (3) variation in contents of sodium and potassium in fillets, (4) variation in proximate composition of nonedible parts, and (5) correlation of special composition characteristics with possible commercial uses.

Variations in physical data. The large variations in size, weight, and fillet yield are of interest (Table 2). In length, the fish varied from 18.7 to 68.7 cm. A comparison of the length with the weight gives some idea of the general condition and quality of the specimen.

The individual fish varied in weight from less than 100 g. to nearly 10 kilograms. When they were divided into 3 groups—large, medium, and small—the group of large-size fish (over 1500 g.) included: buffalo, lake trout, and carp; the group of medium-size fish (1500 to 500 g.) included: northern pike, whitefish, yellow pike, burbot, sheepshead, sucker, and gizzard shad; and the group of small-size fish (under 500 g.) included: Utah chub, squawfish, bass, blue pike, bullhead, yellow perch, lake herring, crappie, mullet, chub, and smelt. The species in each of the above series are listed in decreasing order of average weight.

Fillet yields showed a greater variation than might be anticipated, ranging from 24.5 to 39.3% for those without the skin. The several species for which values above 40% are recorded represent fillets with skins, or weight of the eviscerated fish before removal of the fillets. Usually a higher percentage of fillet can be obtained from the smaller fish, but the actual weight, of course, would be less. In most instances, the fillet weight represents about one-third of the total weight of the fish.

Variation in proximate composition of fillets. The analytical data indicate wide variation in the amount of moisture, oil, protein, and ash among the various species as well as among specimens within a given species (Table 3).[b]

Except for a few deviations, variation in moisture content was small. Most of the values were in the range of 75 to 82%, with an average of 78%. Whitefish, shad, and chub had low values for moisture, whereas crappie, bullhead, and lake herring had high values. High moisture values often were found for the small species, and low values for the larger ones.

[b] Averages, ranges, and factors of variation are used in this paper, owing to the wide variety of specimens included and the large number of factors considered.

TABLE 3

Composition data for fresh-water fish fillets*

Series No.	Quantity	Species	Moisture		Oil		Protein		Ash	
			Av.	Range	Av.	Range	Av.	Range	Av.	Range
			%	%	%	%	%	%	%	%
Central States Area										
1	3	Bass, white	78.8	78.0–79.6	2.27	2.16– 2.40	18.0	17.3–18.7	0.99	0.93–1.04
2	7	Buffalo	77.6	73.6–80.5	4.0	1.6 – 6.1	17.5	16.3–19.0	1.12	1.08–1.19
3	6	Buffalo	75.8		6.2		17.6		1.04	
4	16	Bullhead	81.3	79.0–83.1	1.6	0.8 – 4.4	16.3	15.8–16.7	1.00	0.95–1.12
5	40	Bullhead	82.6		1.7		16.0		1.00	
7	2	Burbot	80.6		1.2		18.0		0.99	
8	16	Carp	78.7	76.4–81.9	3.4	1.8 – 7.3	18.3	17.0–19.3	1.15	0.99–1.29
9	16	Carp	77.0	71.1–83.3	6.3	1.0 –12.5	17.4	15.6–18.6	1.24	0.81–1.27
10	9	Carp	76.9	71.2–79.0	3.5	1.6 – 9.8	18.4	17.4–19.2	1.12	1.06–1.24
11	6	Carp	74.4		7.3		17.4		1.03	
12	16	Chub	76.8	72.2–81.4	8.6	3.9 –12.8	15.1	13.8–17.4	0.92	0.83–1.04
13	16	Chub	77.2	73.7–80.3	7.6	3.3 –10.5	15.1	12.8–17.4	0.96	0.78–1.13
14	7	Chub	72.5		10.8		16.5		1.12	
15	13	Chub	71.2		10.0		16.9		1.09	
16	4	Chub	72.6		9.0		17.8		1.21	
17	17	Chub	74.5		8.7		15.8		1.05	
18	16	Chub	76.3		10.0		12.4		1.01	
19	16	Chub	71.2		13.1		15.5		1.05	
20	9	Crappie	81.8	80.5–82.6	0.8	0.8 – 1.6	16.8	16.3–18.0	0.95	0.93–1.05
21	16	Gizzard shad	67.8	64.8–72.6	14.0	8.8 –17.4	17.2	15.9–17.8	1.22	1.11–1.29
22	16	Herring	81.3	78.0–84.1	1.5	0.8 – 2.9	17.1	15.6–19.5	1.00	0.85–1.13
24	16	Herring	76.8	75.5–78.3	1.8	1.0 – 2.9	20.8	19.6–21.6	1.27	1.18–1.37
25	16	Herring	80.8	78.0–83.6	1.5	0.7 – 3.3	17.9	16.1–19.1	1.07	0.92–1.25
26	16	Herring	77.6	74.9–80.1	1.9	0.9 – 4.3	20.6	19.2–22.1	1.26	1.15–1.36
27	3	Mullet	79.2	77.9–79.8	2.1	1.5 – 2.7	18.6	18.5–18.8	1.15	1.10–1.19
28	11	Mullet (sucker)	81.1	79.7–81.9	1.4	1.2 – 1.9	17.3	16.3–18.3	1.02	0.93–1.11
29	16	Perch, yellow	79.1	78.4–80.1	0.9	0.8 – 1.1	19.8	16.6–20.3	1.26	1.06–1.48
30	16	Perch, yellow	79.5		0.9		18.8		1.16	
31	6	Perch, yellow	81.1		0.8		17.9		0.91	
32	16	Pike, blue	79.9	79.2–81.1	0.9	0.7 – 1.2	19.1	18.2–19.5	1.23	1.10–1.38
33	16	Pike, blue	79.6		1.5		18.6		1.13	
34	16	Pike, yellow	80.0	79.0–81.2	1.3	0.8 – 2.0	19.2	18.7–19.7	1.19	1.12–1.31
35	16	Pike, yellow	79.6		0.8		18.8		1.17	
36	6	Pike, yellow	78.2		1.8		19.8		1.12	
37	10	Pike, northern	80.2	79.6–81.1	1.2	0.7 – 1.7	18.2	17.7–19.0	1.06	1.01–1.08
38	480	Sheepshead (L.)	78.5	74.0–81.7	3.5	1.2 – 8.4	17.5	16.4–18.5	1.03	0.95–1.13
39	155	Sheepshead (R.)	73.9	68.1–78.6	8.0	2.6 –14.8	17.7	16.2–18.9	1.05	0.95–1.18
40	16	Smelt, lake	79.3	77.2–81.7	2.4	1.5 – 3.4	18.5	17.5–20.0	1.06	0.96–1.20
41	12	Trout, lake	72.0	64.3–80.3	10.2	1.9 –19.4	18.1	15.8–19.6	1.03	0.84–1.13
42	3	Trout, lake	70.8	65.3–74.0	9.3	5.2 –14.1	20.0	19.1–20.8	1.15	0.94–1.30
43	15	Trout, lake	52.5	29.8–74.8	36.0	6.7 –63.5	11.3	5.9–17.8	0.53	0.41–1.18
44	16	Whitefish	71.5	61.5–75.9	9.5	4.7 –18.8	18.7	16.3–19.8	1.13	1.04–1.22
45	3	Whitefish	75.1	72.6–78.0	5.4	3.4 – 8.9	18.9	18.3–19.4	1.11	1.00–1.18
46	6	Whitefish	73.7	70.0–78.0	5.9	2.8 – 9.7	19.8	18.4–20.4	1.11	1.03–1.23
Western States Area										
47	16	Smelt, Columbia	79.6	76.5–81.3	6.3	4.6 – 9.0	14.6	13.2–15.3	1.25	0.96–1.40
48	16	Utah chub	79.3		4.8		15.5		1.00	
49	8	Squawfish	77.0	74.3–80.7	4.8	2.1 – 8.6	18.1	17.6–18.4	1.12	1.00–1.38
50	16	Squawfish	80.1		1.8		15.6		1.06	
51	9	Squawfish	79.0		3.1		17.3		0.97	
52	8	Squawfish	78.8		2.4		18.0		1.07	

* If average only is given, a composite sample of the edible part of all fish in the series was made, otherwise the average values represent the averages of all analyses for the individual fish.

Within a given species, variations of as much as 15% were noted. One species, siscowet trout, however, showed a range of 30 to 75% or a variability factor of 2.5 within the 15 specimens analyzed. The maximum variation in moisture was from 29.8% in lake trout, a large species, to 84.1% in lake herring, a very small species. This large factor of variation of 2.8 is explained by the fact that the moisture content varies inversely with the oil content and by the fact that one species of lake trout was found to have a very high content of oil.

In general, oil content and moisture content varied inversely with one another. Most of the species reported in this paper had a range of total content of liquid (moisture plus oil) of between 80 and 83%; the over-all average was close to 81%. The greatest divergence from these values was found in the siscowet trout, in which the total amount of liquid varied from 79 to 93%, which left, in one specimen, not more than 6% of protein.

The greatest variation in a constituent was found in the oil content. Exceptional variation, however, was not the rule. The content of oil in most of the series was less than 5%. Six species averaged more than 5%, and 3 species averaged over 10%. In the species of large size, buffalo and lake trout were high in oil; in the species of small size, burbot, carp, chub, shad, sheepshead, and whitefish were high in oil; and in species having both large- and small-size fish, the smaller fish had the higher oil content. In series of individual species with large ranges in weight, the content of oil was higher in the smaller fish. In burbot, carp, and whitefish, this trend was pronounced. In samples of fish that were taken from both rivers and lakes (sheepshead), the river fish had the higher oil content. The maximum variation in content of oil from all of the fresh-water fish was from 0.7% in northern pike to 63.5% in lake trout. Both species were fairly large fish. This represents an over-all variation factor of 90. For individual species other than siscowet trout, the variation factor did not exceed 10, and it usually was much less than that.

The protein content of the fresh-water fish usually ranged from 16 to 19% and averaged about 17%. All except 3 or 4 of the species were within this range. The lowest values were found for 8 series of chub (103 fish) taken from Lake Michigan during the summer months. The protein content ranged from 12.4 to 17.8%. The lower values usually were found in the smaller-sized fish. In species represented by both river and lake specimens, such as sheepshead, the river fish had the lower content of protein and the higher content of oil. The maximum variation in content of protein, for all specimens, ranged from 5.9% in siscowet trout, a large species, to 22.1% in lake herring, a small species. This 3.7 factor of variation represents extreme cases. In the majority of samples, the factor of variation was not more than 0.2. The maximum for the individual species cited above was 0.5.

Ash content of most fresh-water fish ranged from 0.9 to 1.3% and averaged about 1.1%. One series, however, averaged less than 0.9%. A low value of 0.53% was found in a siscowet trout series. Maximum individual variations ranged from 0.41 in lake trout to 1.48 in yellow perch, giving a factor of variation of 3.6. Species containing a low amount of ash were chub, crappie, yellow perch, and squawfish; those containing a high amount were carp, herring, blue pike, and smelt.

Variation in contents of sodium and potassium in fillets. Since analyses for any of the metal ions have but recently been included in the studies on composition conducted in this laboratory, data are available for only a few of the series. Most of these are reported in another paper *(3)*. The sodium and potassium values were determined for 60 fish from 7 species plus 160 individual sheepshead. There was little correlation between the two minerals or between either of them and any of the other constituents (Table 4).

TABLE 4

Sodium and potassium data for fresh-water fish fillets

Central States Area

Series No.	Quantity	Common name	Sodium		Potassium	
			Av.	Range	Av.	Range
			mg./100 g.	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*
3	6	Buffalo	55		295	
11	6	Carp	48		288	
26	16	Herring	56	46–67	358	301–418
27	3	Mullet	59	46–86	328	326–333
31	6	Perch, yellow	69		223	
36	6	Pike, yellow	50		314	
38	144	Sheepshead (L.)	77	63–95	265	232–309
39	16	Sheepshead (R.)	59	52–71	301	262–323
46	16	Whitefish	51	42–57	297	282–312
Total	219	Averages	58	42–95	297	232–418

(L.)—Lake; (R.)—River.

Sodium occurred in the range of 42 to 95 mg. per 100 g. of fish, the factor of variation was 2.3, and the average value was 58 mg. per 100 g. Potassium occurred in the range 232 to 418 mg. per 100 g. of fish, the factor of variation was 1.8, and the average value was 297 mg. per 100 g.

Variation in proximate composition of the nonedible parts. Composite samples were prepared from all of the nonedible parts of all fish in most of the series. Each of these samples was analyzed for moisture, oil, protein, and ash in order to determine the average composition of the whole fish for each series (Table 5). The ranges in composition and factors of variation are as follows:

Constituent	*Variation Range*	*Factor of Variation*
Moisture	58.0–78.9	1.4
Oil	5.2–27.6	5.3
Protein	12.3–17.8	1.4
Ash	1.7– 9.3	5.5

Chub had the highest values for oil, whereas lake herring had the lowest. This relationship also was found to hold for samples of the edible parts of chub and lake herring. Protein values were just the reverse, being higher for lake herring and low for chub. The highest ash values were found in the large species, buffalo and carp, as well as in the small species, pike and perch. The lowest ash values were found in chub, herring, and squawfish.

TABLE 5

Composition data for fresh-water fish, averages for non-edible parts

Series No.	Quantity	Common name	Moisture	Oil	Protein	Ash
			%	%	%	%
Central States Area						
2	7	Buffalo	70.0	8.9	15.6	7.5
4	16	Bullhead	76.9	5.4	14.9	4.0
5	40	Bullhead	77.6	5.7	14.2	2.7
8	16	Carp	64.9	9.8	17.4	9.3
9	16	Carp	63.8	15.0	16.2	7.1
10	9	Carp	63.3	18.0	14.8	3.6
12	16	Chub	64.6	21.2	12.3	3.6
14	7	Chub	63.0	19.7	14.5	2.8
15	13	Chub	62.6	20.5	14.7	2.6
16	4	Chub	66.1	18.9	13.0	2.8
17	17	Chub	63.0	23.6	12.5	2.1
18	16	Chub	67.6	18.8	13.4	2.8
19	16	Chub	60.0	27.6	12.7	1.9
21	16	Gizzard shad	58.0	23.1	15.1	4.2
22	16	Herring	78.9	5.2	14.4	1.7
23	12	Herring	75.1	9.2	13.0	3.2
24	16	Herring	72.5	6.9	17.8	3.9
25	16	Herring	75.9	6.6	15.5	2.5
26	16	Herring	72.2	7.9	17.4	2.8
28	11	Mullet, sucker	73.5	7.8	14.5	4.4
29	16	Perch, yellow	68.5	9.2	17.7	7.3
30	16	Perch, yellow	68.8	8.7	16.3	7.5
32	16	Pike, blue	69.6	7.9	17.8	6.2
33	16	Pike, blue	66.8	10.7	15.9	7.0
34	16	Pike, yellow	70.6	7.5	17.6	5.6
35	16	Pike, yellow	71.2	8.9	16.3	5.1
37	10	Pike, northern	71.8	5.3	16.5	4.7
41	12	Trout, lake	64.3	16.0	15.6	4.7
42	3	Trout, lake	64.1	20.2	15.6	1.9
44	16	Whitefish	62.1	19.4	16.4	3.1
46	6	Whitefish	63.1	17.6	15.7	3.1
Western States Area						
48	16	Utah chub	64.3	20.2	13.1	3.3
50-52	33	Squawfish	69.5	10.4	16.0	5.9

Correlation of special composition characteristics with possible commercial uses. According to our present knowledge of nutrition, the most desirable product for human consumption would be a specimen with high content of protein (over 17%), low content of oil (under 5%), and low content of sodium (under 60 mg. per 100 g.). It should be at least of medium size in order to be economical to handle.

Of the species reported here, the following are commonly used for food: bass, bullhead, perch, pike, smelt, trout, whitefish, and to a lesser extent—carp, herring, and sheepshead. Pike and whitefish are medium in size and have low values for oil and sodium, as well as high values for protein. Of the smaller fish, bass and yellow perch have similar low values as do carp, herring, and sheepshead taken from rivers. If a high content of oil and low content of protein is desired, river smelt or siscowet trout are the best choices.

For animal food, the entire fish commonly is used. Composition data for the whole fish have been calculated (Table 6) for those species for which sufficient data are available, except in two instances where the whole fish was analyzed.

TABLE 6

Composition data for fresh-water fish, averages calculated for whole fish

Series No.	Quantity	Common name	Moisture	Oil	Protein	Ash
			%	%	%	%
Central States Area						
2	7	Buffalo	72.6	7.3	16.3	5.4
4	16	Bullhead	77.8	4.7	15.2	3.4
6	9	Burbot*	76.9	7.0	14.3	1.6
8	16	Carp	68.8	7.9	17.6	6.9
9	16	Carp	68.7	12.6	16.5	5.4
10	9	Carp	67.3	14.7	15.8	3.1
14	7	Chub	66.0	16.8	15.4	2.3
15	13	Chub	65.4	17.0	15.5	2.1
16	4	Chub	68.3	15.6	14.6	2.3
17	17	Chub	66.6	19.0	13.4	1.8
19	16	Chub	63.9	22.5	13.7	1.6
21	16	Gizzard shad	61.2	20.1	15.8	3.2
22	16	Herring	78.8	3.7	15.6	1.9
24	16	Herring	74.1	5.0	18.9	2.9
25	16	Herring	77.7	4.7	16.4	1.9
26	16	Herring	74.1	5.8	18.5	2.3
29	16	Perch, yellow	72.7	5.9	18.5	5.0
30	16	Perch, yellow	72.2	6.1	17.1	5.4
32	16	Pike, blue	74.1	4.8	18.4	4.0
33	16	Pike, blue	71.2	7.5	16.8	5.0
35	16	Pike, yellow	74.1	6.1	17.1	3.7
37	10	Pike, northern	74.0	4.2	16.9	3.7
44	16	Whitefish	66.5	14.8	17.5	2.2
Western States Area						
49	8	Squawfish	71.9	8.6	16.7	4.4

* Samples prepared from whole fish.

These data were obtained as follows: the fillet composition values were multiplied by the percent fillet yield. The percentage of nonedible part represented 100% minus percent fillet yield. The composition values for the nonedible part were multiplied by this percentage. The sum of the two values thus obtained gives the calculated composition of the whole fish. Most of these species represent edible fish and thus would be too expensive to feed to animals. The data do indicate, nevertheless, the possibility of selecting species having the desired composition ratios for a particular purpose.

SUMMARY

Twenty-one species of fresh-water fish have been analyzed for their proximate composition, and eight of these species have been analyzed also for their content of sodium and potassium. In general, the composition of these fish was uniform. The exceptions, however, demonstrate the wide variation in composition that sometimes exists both among the different species of fish and among the individual fish within a single species.

From the data collected, it is possible to choose species of fresh-water fish that fit either into a normal diet or into a variety of special-purpose diets.

The investigation of the influence on composition of size of fish, season of capture, and area of capture is still in progress and will be reported in later publications.

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CHEMICAL CHANGES ASSOCIATED WITH THE RIPENING OF APPLES AND TOMATOES[a]

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The ripening and eventual softening of fruits is a natural phenomenon. More knowledge concerning the chemical changes associated with ripening is necessary to improve the present handling techniques for maintaining the quality of fruits in fresh market channels. In this study, the chemical changes of several constituents in whole apples and tomatoes were ascertained at the unripe, ripe, and overripe stages of maturity to help explain the softening process.

Objective measurements of fruit quality have as their goal the establishment of precise specifications to characterize any given lot of material. For example, measurements such as alcohol-insoluble solids, crude fiber, protein, acid content, and fruit pressure tests have been used by many workers for quantitative evaluation of the nutrition and quality levels of fruits and vegetables *(3, 5, 10, 11, 12, 18, 20, 21, 26)*. For many years, changes in the quantity and type *(16)* of pectic substances have been considered to play a role in ripening and firmness of fruits *(7, 13, 15, 19, 22, 25, 27, 28)*. Some evidence suggests that the pectic components of fruits may be hydrolyzed to soluble forms, with accompanying demethylation, and finally to smaller, less complex, units *(4)*. The mechanism involved in the transformation of these pectic substances as fruits mature, as well as their function in providing firmness to plant tissues, has not been clearly demonstrated. Older methods for extracting and analyzing pectic substances from plant material have been not entirely free from criticism *(15)*. Recent methods *(24)* use less drastic extractions with little or no degradation, possibly yielding a product comparable to pectin *in situ*.

This study seeks to provide fundamental information, using reliable methods, for a quantitative evaluation of some chemical changes associated with the maturation process of apples (Red Delicious and Stayman) and tomatoes (Brookston and Valiant). Especial attention is given to the determination and characterization of the pectic constituents.

EXPERIMENTAL MATERIALS AND METHODS

Apple and tomato fruits. The fruits were obtained from orchards and plots at the University Farm during the 1953 and 1954 seasons. They were carefully harvested at the unripe and ripe stages of maturity with the ripe stage allowed to become overripe in the laboratory. Extremely large and small specimens were excluded as well as those with cracks or blemishes. The selection of fruits for analyses at the 3 stages of maturity was on the basis of skin color and firmness. Unripe fruits were full size but essentially green and were hard. Ripe fruits had full color change and were firm. Overripe fruits were similar to ripe, except senescence and softening had taken place, hence special care was required in handling them.

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Fruit	Moisture and titratable acidity		Alcohol-insoluble fractions	
	No. of samples	Approx. size of each sample (g.)	No. of samples	Approx. size of each sample (g.)
Apples (Red Delicious)	5	500	10	1000
Apples (Stayman)	3	500	10	1000
Tomatoes (Brookston)	2	1000	10	1000
Tomatoes (Valiant)	5	500	9	1000

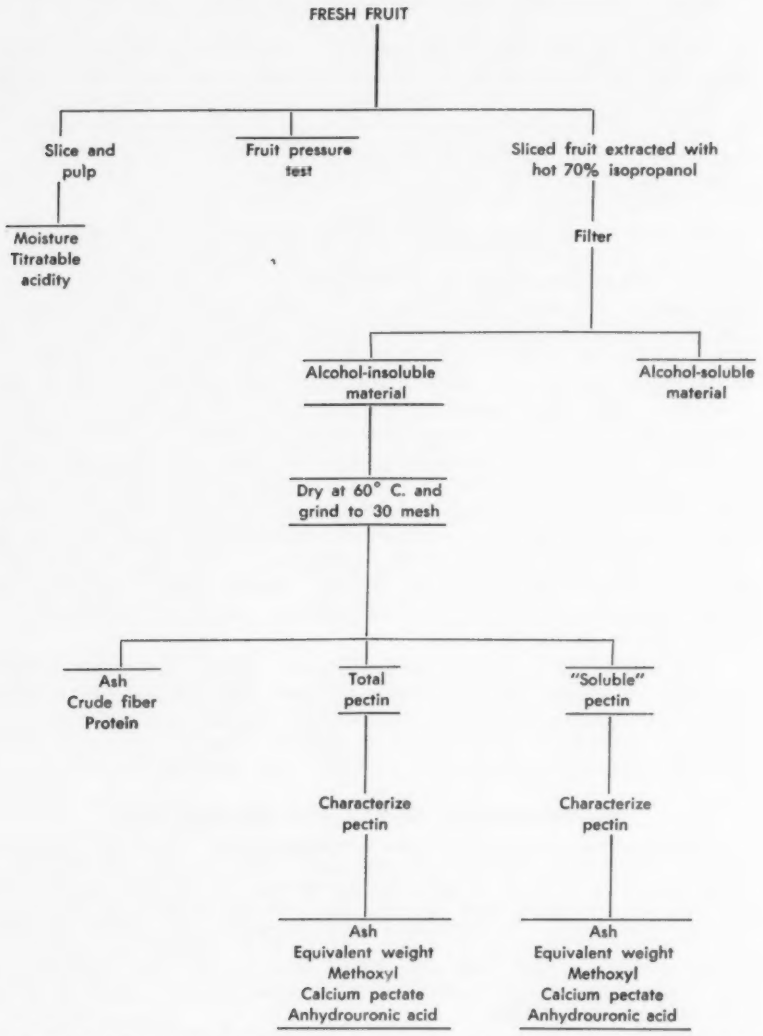

Figure 1. Flowsheet for schematic analyses of unripe, ripe, and overripe apples and tomatoes.

At the 3 stages of ripeness, the fruits were washed, surface air dried, stems removed and sampled at random for analyses according to the flowsheet, Figure 1. The fruits were divided for the phases of analyses into the following initial samples:

Analyses of the fresh fruits. Following the scheme in Figure 1, the fresh fruit samples were analyzed promptly.

Fruit pressure test. Two Magness-Taylor pressure testers *(20)* equipped with 7/16-inch diameter plungers and having ranges of 0-30 pounds (for apples and unripe tomatoes) and 0-10 pounds (for ripe and overripe tomatoes) were used to determine firmness by reading the force necessary for the plunger to penetrate the fruit 5/16 inch on a peeled area. An average pressure test value from fifteen fruit was obtained for each sample at the 3 stages of ripeness. Pressure tests were made on the sides of apples and on the stylar end of tomatoes.

Moisture and titratable acidity. A homogeneous sample was prepared for analysis by slicing the fresh fruit and pulping in a Waring blender. Two 25-g. portions of each blended sample were dried to constant weight at 70° C., for approximately 24 hours in order to determine the moisture. The titratable acidity was determined in duplicate by the A.O.A.C. method for fruits *(1)* on a 25-g. blended portion of each sample. The results were expressed as milliequivalents of acid per 100 g. of material.

Alcohol-soluble and alcohol-insoluble fractions. Approximately one-kilogram of the various fruit samples were sliced (except for overripe tomatoes which were crushed by hand) into a measured amount of boiling 99% isopropanol so that the final mixture contained 70% by volume of isopropanol. The mixture was boiled for 5 minutes, allowed to cool overnight, and filtered through muslin. The alcohol-insoluble material was pulped by passing thru a food chopper and dehydrated by 4 repeated washings with 99% isopropanol. The insoluble material was dried at 60° C. overnight, ground to pass a 30-mesh screen, and stored in bottles for subsequent analysis. The constituents in the initial alcohol-soluble fractions were considered in another study *(23)*.

The alcohol-insoluble fractions obtained from the 10, approximately one-kilogram samples of fruits at the 3 stages of maturity were combined after weighing into two-composite samples so as to provide sufficient representative material for pectin extractions, ash, crude fiber, and protein analyses.

The composite samples of the alcohol-insoluble materials were analyzed in duplicate for ash, crude fiber, and protein according to A.O.A.C. methods *(1)*. Pectic substances were extracted from the composite samples by essentially the methods of Owens and co-workers *(24)*. The extractions of total and so-called cold-extracted pectins (designated in this article as "soluble" pectin) were made on twenty-five grams of material in each case, using Calgon to aid the extraction of pectic substances, and acid-isopropanol instead of ethanol to precipitate the two types of pectins. Starch was removed before precipitation by enzymatic digestion. The precipitated pectins were dried overnight at 60° C., ground to pass a 30-mesh screen, and stored in bottles for subsequent characterization.

Characterization of total and "soluble" pectins. The ash, apparent equivalent weight, and anhydrouronic acid contents were determined by methods described by Owens and co-workers *(24)*. The saponification method of von Fellenberg *(2,9)* was used to determine the methoxyl content. The Emmett and Carré *(8)* modification of the Carré and Haynes method *(6)* was employed for the determination of the calcium pectate content. The procedures for the analyses of apparent equivalent weight, methoxyl, and calcium pectate contents were adapted so as to follow one another on the same pectin samples.

RESULTS AND DISCUSSION

Composition of unripe, ripe, and overripe fruits and their 70% alcohol-insoluble fractions. Tables 1 and 2 show some of the chemical changes associated with the ripening of apples (Red Delicious and Stayman) and tomatoes (Brookston and Valiant). The moisture content of the fresh fruits showed no significant change during the maturation process with very little difference in composition between the varieties of apples and tomatoes. The titratable acidity decreased substantially between the ripe and overripe stages of the fresh fruits with significance in all varieties

TABLE 1

Composition of unripe, ripe, and overripe apple varieties and their 70% alcohol-insoluble fractions (Moisture-free basis)

	Composition		Unripe	Ripe	Overripe	L.S.D. .05	L.S.D. .01
Fresh fruit	Moisture (%)	a [1]	83.4	82.9	83.8	N.S.	N.S.
		b	86.9	85.4	85.4	N.S.	N.S.
	Titratable acidity (m.e./100 g.)	a	27.17	22.64	16.26	1.05	1.53
		b	60.48	67.81	34.89	7.05	11.68
	Alcohol-insoluble solids (%)	a	36.04	29.98	16.75	2.34	3.41
		b	41.41	32.11	16.68	2.11	3.07
	Fruit pressure test (lbs.)	a	—	—	—	—	—
		b	17.77	15.52	7.82	0.93	1.55
70% Alcohol-insoluble fractions	Ash (%)	a	1.79	1.81	2.93	0.12	0.19
		b	1.34	1.74	2.13	0.05	0.08
	Crude fiber (%)	a	14.34	15.42	30.47	0.52	0.79
		b	14.27	17.17	31.38	0.85	1.29
	Protein (%)	a	3.88	4.18	7.26	0.15	0.22
		b	3.48	3.50	7.71	0.21	0.32
	Total pectin (%)	a	10.74	10.99	17.81	3.47	N.S.
		b	12.57	12.60	15.96	1.46	N.S.
	"Soluble" pectin (%)	a	4.43	4.86	9.64	0.53	1.21
		b	6.07	7.32	10.46	0.78	1.80
	"Soluble" pectin / Total pectin (%)	a	41.2	44.2	54.1	—	—
		b	48.3	58.1	65.5	—	—

[1] a = Red Delicious apples; b = Stayman apples.

at the 5% level. This change in acidity was a confirmation of Luh and co-workers *(19)* and Saywell and Cruess *(26)* on tomatoes. It was suggested by Smock and Neubert *(27)*, after describing a similar trend with apples, that the acids possibly serve along with sugars as a partial substrate for respiration. Alcohol-insoluble solids decreased considerably during the ripening of the apples with significance among the 3 stages of maturity at the 1% level. With Brookston tomatoes, a significant decrease at the 1% level was observed between the unripe to ripe and unripe to overripe stages, while Valiant tomatoes showed practically no change in alcohol-insoluble solids. It is generally considered that the alcohol-insoluble fractions includes starch, protein and cell wall constituents such as pectin, hemicellulose, and cellulose. The decrease in this fraction as fruits ripen may be attributed to a conversion of insoluble to soluble material. The fruit pressure tests, which

TABLE 2

Composition of unripe, ripe, and overripe tomato varieties and their 70% alcohol-insoluble fractions (Moisture-free basis)

	Composition		Unripe	Ripe	Overripe	L.S.D. .05	L.S.D. .01
Fresh fruit	Moisture (%)	a[1]	92.2	92.0	92.3	N.S.	N.S.
		b	92.4	92.6	92.9	N.S.	N.S.
	Titratable acidity (m.e./100 g.)	a	85.26	104.38	83.51	11.33	N.S.
		b	120.03	94.81	88.05	16.12	23.45
	Alcohol-insoluble solids (%)	a	33.95	25.48	25.89	1.31	1.91
		b	26.96	26.54	27.60	N.S.	N.S.
	Fruit pressure test (lbs.)	a	23.03	1.95	0.90	3.87	6.42
		b	—	—	—	—	—
70% Alcohol-insoluble fractions	Ash (%)	a	5.89	7.25	7.84	0.25	0.39
		b	6.14	7.37	8.00	0.30	0.70
	Crude fiber (%)	a	21.25	29.75	29.32	0.86	1.31
		b	24.39	26.90	27.20	N.S.	N.S.
	Protein (%)	a	16.03	20.61	22.08	0.83	1.25
		b	22.30	23.30	23.88	N.S.	N.S.
	Total pectin (%)	a	12.85	16.54	15.83	N.S.	N.S.
		b	13.03	13.43	12.10	0.19	N.S.
	"Soluble" pectin (%)	a	9.25	12.77	13.53	2.62	N.S.
		b	8.54	8.74	8.71	N.S.	N.S.
	"Soluble" pectin / Total pectin (%)	a	72.0	77.2	85.5	—	—
		b	65.5	65.1	72.0	—	—

[1] a = Brookston tomatoes; b = Valiant tomatoes.

substantiate the decrease in firmness as fruits mature, were found to be significant for all stages of ripeness of Stayman apples at the 1% level but for Brookston tomatoes only at the unripe to ripe or unripe to overripe stages. The primary usefulness of this test is to indicate differences in firmness between two or more lots of the same fruit variety on a certain date or to indicate the general degree of firmness *(27)*.

The analyses of the 70% alcohol-insoluble fractions (Tables 1 and 2) of the fruits include the ash (inorganic material), the crude fiber (essentially hemicellulose and cellulose), the protein (total $N \times 6.25$) and the pectic substances. It is of interest to note in this fraction that a total of the above constituents for which analyses were made accounted for an average of approximately 31% at the unripe stage to 58% at the overripe stage with the two varieties of apples and 61% to 73% with the tomato varieties,

respectively. In general, the analyses of the 70% alcohol-insoluble fractions showed an apparent increase with the ripening of the fruits in ash, crude fiber, proteins, and pectic substances with statistical significance in some instances at either the 1% or 5% levels. The ratio of "soluble" pectin to total pectin was found to be higher for tomatoes than apples and to increase from the unripe to overripe stages for both fruits.

The changes associated with the ripening of total and "soluble" pectins, when calculated on the original fruit basis, may be observed in Figure 2. Expressing the two forms of pectins on the original fruit basis perhaps more closely indicates the total content of pectic substance in the fruits. Although it has been shown *(15)* that the ripening of these fruits was associated with a decrease in total pectin and an accompanying increase in "soluble" pectin, the data plotted in Figure 2 do not entirely confirm these findings. The total pectin decreased from the unripe to overripe stages for the fruits. The trend was found statistically significant at the 1% level for Stayman apples (L.S.D. = 1.08) and Valiant tomatoes (L.S.D. = 0.03).

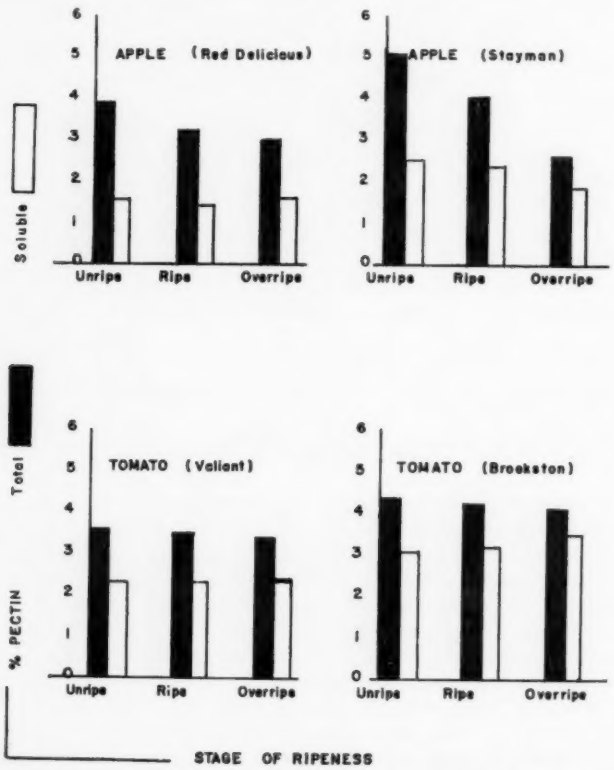

Figure 2. Changes with ripeness of total and "soluble" pectins of original fruits as calculated from 70% alcohol-insoluble fractions (Moisture-free basis).

There was no consistent trend for "soluble" pectin. Kertesz and McColloch *(17)* working on changes of pectic substances in mature John Baer tomatoes reported that there were no trends in the pectic changes as the tomatoes matured. No consistent trends could be observed when the crude fiber and protein data were converted to a whole fruit basis.

Characterization of the total and "soluble" pectins of apples and tomatoes. The results of the characterization of total and "soluble" pectins extracted from 70% alcohol-insoluble fractions of apples and tomatoes are presented in Table 3 and 4 respectively. In order to provide a more precise and accurate presentation of the data the results are expressed on a moisture- and ash-free basis. The analyses show that ripening of the fruits in this study was associated with a decrease in the apparent equivalent weight and in the degree of esterification especially for tomatoes. These changes in the composition of the pectins were significant in many instances at the 5% and 1% levels (see Tables 3 and 4). The findings suggest that during the maturation process, some of the ester linkages in the pectins may be hydrolyzed to carboxyl groups leading to a decrease in the equivalent weight. The methoxyl contents of the apple pectins at the three stages of ripeness were higher than those of the tomato varieties. The high enzyme activity of pectin-methylesterase in tomatoes as compared to apples may account for this observation *(14, 15)*. In order to correct for the calcium in calcium pectate the latter was calculated to pectic acid. The calculated pectic acid and anhydrouronic acid contents compare quite well and indicate the uronide contents of the pectins as well as their trend to increase slightly during fruit maturation. There is a general indication that the "soluble" pectins are slightly higher than the total pectins in anhydrouronic acid and calculated pectic acid contents. It is possible that during the extraction of the "soluble" pectins there was less degradation of pectic substances than with the total pectins.

Effect of storage temperatures on changes of constituents in ripe Wiltmaster tomatoes. A study was made to determine the effect of storage temperature upon the maintenance of firmness and chemical changes of ripe tomatoes (Wiltmaster) held for 4 days at cool storage (35-40° F.) as compared with tomatoes held in the laboratory (84-87° F.). The spoilage of ripe tomatoes held 4 days at cool storage prior to sampling was about 5% with little change in firmness while those held at air temperature softened considerably, requiring care in handling and about one-third spoiled. The various chemical changes of the tomatoes, determined by sampling and analyzing according to the scheme in Figure 1, are shown in Table 5. Although the data are expressed on a moisture-free basis, they can be easily calculated to the fresh fruit basis, since no significant changes in moisture content were found at the different storage temperatures. The titratable acidity varied among the tomato varieties at the ripe stage from 95 (Valiant), 104 (Brookston), to 110 (Wiltmaster) milliequivalents of acid per 100 g. of fruit as shown in Tables 1, 2, and 5. The titratable acidity not only varies from variety to variety but the data in Table 5 indicate a significant (1% level) decrease in the acidity of ripe tomatoes held for 4 days at 84-87° F. This trend is accompanied by a softening of the fruit as indicated by the fruit pressure tests. There was no significant change in the total and "soluble" pectins as calculated for the original tomatoes from the 70%

TABLE 3

Characterization of total and "soluble" pectins extracted from 70% alcohol-insoluble fractions of apple varieties (Moisture- and ash-free basis)

Character		Unripe		Ripe		Overripe		L. S. D.			
								.05		.01	
		Total	"Soluble"	Total	"Soluble"	Total	"Soluble"	Total	"Soluble"	Total	"Soluble"
Ash (%)	a [1]	0.97	2.66	1.24	1.73	0.86	1.70	—	—	—	—
	b	1.13	1.60	1.26	1.69	1.60	1.46	—	—	—	—
Apparent equivalent weight	a	1257	1076	1209	1045	965	895	77	51	117	77
	b	1529	1087	1235	1052	992	866	48	21	73	31
Methoxyl (%)	a	8.50	8.98	8.87	9.00	8.62	9.26	N.S.	0.20	N.S.	N.S.
	b	7.79	9.20	9.26	9.39	9.47	9.74	0.15	0.31	0.23	N.S.
Calcium pectate (%)	a	82.9	87.9	85.7	88.1	89.2	93.5	4.4	0.8	N.S.	1.2
	b	75.9	88.2	86.6	90.4	91.4	96.4	1.6	2.1	2.4	3.2
Anhydrouronic acid (%)	a	78.5	83.0	79.4	83.1	81.4	89.3	N.S.	N.S.	N.S.	N.S.
	b	59.1	73.4	70.3	77.6	74.1	81.1	1.9	4.1	2.9	N.S.
Calcium pectate calculated as pectic acid [2] (%)	a	74.8	79.3	77.3	79.5	80.5	84.4	—	—	—	—
	b	68.5	79.6	78.2	81.6	82.5	87.0	—	—	—	—
Methoxyl content expressed on 100% AUA basis (%)	a	10.83	10.82	11.17	10.83	10.59	10.37	—	—	—	—
	b	13.18	12.53	13.17	12.10	12.78	12.01	—	—	—	—
Degree of esterification (16.3 = 100%) (%)	a	66.4	66.4	68.5	66.4	65.0	63.6	—	—	—	—
	b	80.9	76.9	80.8	74.2	78.4	73.7	—	—	—	—

[1] a = Red Delicious apples; b = Stayman apples.

[2] (Calcium pectate) $\left(\frac{176}{195}\right)$ = pectic acid

TABLE 4

Characterization of total and "soluble" pectins extracted from 70% alcohol-insoluble fractions of tomato varieties (Moisture- and ash-free basis)

Character		Unripe		Ripe		Overripe		L.S.D.			
								.05		.01	
		Total	"Soluble"	Total	"Soluble"	Total	"Soluble"	Total	"Soluble"	Total	"Soluble"
Ash (%)	a [1]	1.84	2.72	3.22	4.32	3.11	4.96	—	—	—	—
	b	4.64	5.21	4.24	7.52	4.79	7.32	—	—	—	—
Apparent equivalent weight	a	810	791	655	605	539	520	39	31	59	47
	b	789	730	631	566	546	498	93	12	N.S.	28
Methoxyl (%)	a	8.10	8.33	7.45	7.60	7.07	6.68	0.18	0.19	0.27	0.29
	b	7.01	7.53	6.15	6.58	5.59	5.89	0.61	0.47	N.S.	1.09
Calcium pectate (%)	a	89.2	89.5	93.9	94.8	95.2	96.6	2.4	1.8	3.7	2.8
	b	86.1	86.8	87.3	92.4	88.8	94.4	N.S.	3.7	N.S.	N.S.
Anhydrouronic acid (%)	a	74.8	80.4	80.0	86.0	83.3	85.8	2.3	0.9	3.5	1.4
	b	78.1	83.1	80.8	86.9	85.0	86.1	N.S.	0.9	N.S.	2.2
Calcium pectate calculated as pectic acid [2] (%)	a	80.5	80.8	84.8	85.6	85.9	87.2	—	—	—	—
	b	77.7	78.3	78.8	83.4	80.1	85.2	—	—	—	—
Methoxyl content expressed on 100% AUA basis (%)	a	10.83	10.36	9.31	8.84	8.48	7.79	—	—	—	—
	b	8.98	9.06	7.61	7.57	6.58	6.84	—	—	—	—
Degree of esterification (16.3 = 100%) (%)	a	66.4	63.6	57.1	54.2	52.0	47.8	—	—	—	—
	b	61.3	55.6	46.7	46.4	40.4	42.0	—	—	—	—

[1] a = Brookston tomatoes; b = Valiant tomatoes.

[2] (Calcium pectate) $\left(\frac{176}{195}\right)$ = pectic acid

TABLE 5

Effect of storage temperatures on changes of constituents in ripe Wiltmaster tomatoes (Moisture-free basis)

	Constituents		Fresh ripe	Ripe — held 4 days in cool storage (35-40° F.)	Ripe — held 4 days at (84-87° F.)	L.S.D. .05	L.S.D. .01
Fresh fruit	Moisture	(%)	92.6	92.7	92.8	N.S.	N.S.
	Titratable acidity (m.e./100 g.)		109.57	118.60	93.70	8.92	14.80
	Alcohol-insoluble solids	(%)	25.35	28.21	27.22	N.S.	N.S.
	Fruit pressure test (lbs.)		1.17	1.22	1.06	N.S.	N.S.
Constituents in original fruit as calculated from 70% alcohol-insoluble fraction	Ash	(%)	1.84	2.19	2.19	0.05	0.08
	Crude fiber	(%)	6.68	8.05	7.97	0.34	0.52
	Protein	(%)	5.59	6.18	5.92	0.07	0.10
	Total pectin	(%)	3.58	3.85	3.45	N.S.	N.S.
	"Soluble" pectin	(%)	3.00	2.83	2.97	N.S.	N.S.

alcohol-insoluble fractions. The methoxyl contents of the extracted total pectins decreased from 8.04% at the fresh ripe stage, to 7.90% after 4 days of cool storage, to 7.45% after 4 days at 84-87° F. with significance between the cool and warm storage at the 1% level (L.S.D. = 0.25). These pectins remained constant in anhydrouronic acid contents being 82.5%, 82.2%, and 82.2% respectively. These results indicate that during the warm storage of tomatoes the demethylation of the pectin is accelerated while cool storage tends to retard demethylation.

In order to understand the process, considerably more basic information must be obtained on the chemical changes associated with ripening of these fruits. Information on some of the constituents of the 70% alcohol-soluble fractions are covered in another publication *(23)*. Further knowledge of other polysaccharides in the alcohol-insoluble fractions of these fruits such as starch, sugars, cellulose, and hemicellulose may indicate a role in maintaining firmness and other attributes of good quality.

SUMMARY

Certain constituents in fresh apples (Red Delicious and Stayman varieties) and tomatoes (Brookston and Valiant varieties) were determined at the unripe, ripe, and overripe stages of the fruits to disclose any chemical changes associated with ripening.

In the fresh fruits the titratable acidity decreased substantially between the ripe and overripe stages with significance at the 5% level. The fruit pressure tests (performed only on Stayman apples and Brookston tomatoes) substantiated the decrease in firmness as fruits mature and were found to

be significant for all stages of ripeness of Stayman apples at the 1% level but for Brookston tomatoes only at the unripe to ripe or unripe to overripe stages.

The 70% alcohol-insoluble solids decreased considerably during the ripening of the apples with significance at the 1% level. With Brookston tomatoes, a significant decrease at the 1% level was observed at either the unripe to ripe or unripe to overripe stages while Valiant tomatoes showed little change. On a fresh weight basis, the total pectin decreased significantly from the unripe to overripe stages for the Stayman apples and Valiant tomatoes, while for "soluble" pectin, crude fiber, and protein, no consistent trends could be observed for any of the fruits. For Red Delicious apples and Brookston tomatoes, the total pectin decreased, but without significance.

A characterization of the total and "soluble" pectins showed that ripening of the fruits was associated with a decrease in the apparent equivalent weight and in the degree of esterification. The pectic acid calculated from the calcium pectate contents and the anhydrouronic acid contents compared quite well and indicate the uronide content of the pectins as well as the trend to increase slightly during fruit maturation.

Spoilage of ripe, Wiltmaster tomatoes for 4 days in cool storage (35-40° F.) was about 5%, in warm storage (84-87° F.) about 33%. Cool storage of ripe tomatoes retarded the loss of the titratable acidity and slowed the demethylation of extracted pectic substances.

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THE VITAMIN AND AMINO ACID CONTENT OF DRIP OBTAINED UPON DEFROSTING FROZEN PORK[a]

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The most obvious change occurring upon thawing frozen meat is the exuding of a blood-like fluid commonly called drip. Although Cook, Love, Vickery and Young *(3)* reported over 30 years ago that drip contained about 9% protein, little effort has been made to determine the components of drip until recently. Pearson, Burnside, Edwards, Glasscock, Cunha and Novak *(11)* found that drip from frozen defrosted beef contained a considerable amount of the various B-complex vitamins, and Seagran *(14)* recently reported a marked similarity between the protein fractions of fresh fish muscle and that of drip collected upon thawing frozen fish.

This study was undertaken to ascertain the amount of certain B-complex vitamins and amino acids in drip obtained upon thawing frozen pork.

EXPERIMENTAL

A pair of wholesale pork loins was obtained and the *Longissimus dorsi* muscle removed and cut into chops ¾ in. thick. The chops were packaged individually in freezer paper, frozen and stored at approximately −18° C. until removed at random for analysis. A section was removed and weighed from one frozen chop and immediately prepared for analysis by making a meat-water slurry in the Waring blender. The other chop was thawed on a wire screen inside a large funnel, which drained into a graduate cylinder. The entire apparatus for collecting drip was located inside a closed 50 lb. lard can, which had a thin layer of water on the bottom to saturate the air and was located in a cooler held at approximately 2° C. Approximately 18–20 hrs. were allowed for thawing. This procedure was followed since earlier studies *(18)* had shown that these thawing conditions resulted in more repeatable results for collection of drip. A drop or two of toluene was placed in the graduate cylinder to prevent microbial growth. The chop was weighed, both before and after thawing, and the drip was measured to the nearest 0.1 ml.

Both the drip and meat were hydrolyzed by the same method for each vitamin or amino acid in order to remove the possibility of variation due to differennces in treatment. The level of each vitamin or amino acid was determined by either microbiologcal or chemical methods for both the drip and meat.

Thiamine was determined by the thiochrome method *(2, 8)* as modified by the Association of Vitamin Chemists, Inc. *(1)*. Riboflavin was measured microbiologically as outlined in the Difco Manual *(4)* after hydrolysis by heating with 0.1 N. HCl. Niacin was assayed by acidimetric determination of the growth response of *Lactobacillus arabinosus* 17–5 *(4, 15, 16)*. Vitamin B_{12} was determined with *Lactobacillus leichmannii (4, 9)* after hydrolyzing a buffered homogenate in the autoclave by the procedure of Scheid and Schweigert *(12)*. Pantothenic acid was measured with *Lactobacillus arabinosus* after hydrolyzing with intestinal phosphatase and Dowex-1 treated hog kidney enzyme *(4, 10)* to liberate the vitamin. Pyridoxine, pyridoxamine and pyridoxal were determined with the fluorometric method developed by Japanese workers *(5, 6, 7)*.

Leucine, isoleucine, lysine and methionine were measured microbiologically with *Leuconostoc mesenteriodes* P-60 after acid hydrolysis *(4, 9, 17)*. The method of Difco Laboratories *(4)* specified the use of a D-L standard for both isoleucine and methioninne,

[a] Journal Article 2347, Michigan Agricultural Experiment Station, East Lansing, Michigan.

thus, it was necessary to correct the values, as the test organism responds only to the L-form *(13)*. It was also necessary to correct the L-tryptophan standard for the racemization to the DL-isomer during NaOH hydrolysis with heat. Similarly, the lysine standard was made on the hydrochloride form, and thus values were corrected to the pure L-isomer. The tissue was freeze dehydrated prior to preparation for analysis but the drip was treated directly. Tryptophan was hydrolyzed by autoclaving in NaOH, to which L-cysteine was added to prevent oxidative destruction *(9)*, and determined by acidimetric measurement of the growth response of *Lactobacillus arabinosus* 17-5 *(4, 15)*.

The total amount of each vitamin and amino acid was calculated for both the drip and the frozen meat, and the percentage of each found in the drip was computed.

RESULTS

Volume of drip. The percentage of drip obtained for each analysis is contained in Table 1. Examination of the data shows great variation in the percentage of drip lost on thawing the various chops, with extreme ranges of 6.35 to 12.40%. However, it is obvious from the data that percentage losses of the different nutrients in drip varied considerably even when the assay was made on the same sample of drip. For example, both riboflavin and niacin were determined on the same drip collection (drip loss of 9.23% by weight), and yet losses for riboflavin were less than half as large as niacin.

Since each analysis was made on a single collection of drip, with the exception of those where several amino acids or vitamins were determined on the same sample, it is not possible to determine the influence of volume of drip upon the concentration of the various nutrients.

B-complex vitamins. A summary of the amount of the various B-complex vitamins in both the meat and drip and the percentage lost in the drip is presented in Table 1.

TABLE 1

B-complex vitamins and amino acids in frozen meat and drip, and the percentage of the total lost in the drip[1]

Vitamin or amino acid	Wt. of meat gm.	Meat μg./gm.	Vol. of drip ml.	Drip μg./ml.	Percentage drip	Percentage of total lost in drip
Thiamine	182.0	7.4	15.0	8.1	8.24	9.02
Riboflavin	146.3[3]	3.20	13.5	1.44	9.23	4.15
Niacin	146.3[3]	96.7	13.5	122.0	9.23	10.69
Vitamin B_6[2]	244.1	3.88	15.5	5.32	6.35	8.71
Pantothenic acid	181.9	11.34	16.0	8.96	8.80	6.95
Vitamin B_{12}	169.0	0.000336	12.5	0.00023	7.40	5.06
Isoleucine	174.2[4]	10,938	21.6	9,771	12.40	11.08
Leucine	174.2[4]	17,670	21.6	13,840	12.40	9.71
Lysine	174.2[4]	20,202	21.6	14,016	12.40	8.60
Methionine	174.2[4]	5,083	21.6	3,107	12.40	7.58
Tryptophan	201.2	2,060	19.5	1,520	9.69	7.15

[1] Each value represents a single analysis.
[2] Total Vitamin B_6 = Pyridoxine + Pyridoxamine + Pyridoxal.
[3] Same sample used for drip analyses for niacin and riboflavin.
[4] Drip analyses for isoleucine, leucine, lysine and methionine made on same sample.

Calculations of the percentage of thiamine in the drip showed that 9.02% of the total in the meat had leached out with the drip (Table 1). This value for the drip from frozen pork is about 3% lower than values reported for drip from frozen beef *(11)*. The riboflavin content of the frozen pork ribeye was 3.20 μg. per g. and 1.44 μg. per ml. for the drip (Table 1). Thus, the drip contained less than half as much riboflavin per unit of weight as the meat, and the percentage lost in the drip amounted to only 4.15%. A comparison with the data for beef *(11)* shows that pork drip on a percentage basis contained less than half as much of the total riboflavin. Although 10.69% of the niacin was lost in the drip, this is approximately 5% less than was reported for beef by Pearson *et al. (11)*.

TABLE 2

The amount and percent of pyridoxine + pyridoxamine and pyridoxal found in the meat and drip

	Pyridoxine + Pyridoxamine	Pyridoxal
Meat—frozen—total amt. in gammas	112.54	390.70
Drip—total amt. in gammas	18.64	23.92
Percentage lost in drip	16.56	6.12

Total vitamin B_6 is reported in Table 1, and a breakdown into pyridoxal and pyridoxine plus pyridoxamine is found in Table 2. The meat contained 3.88 μg. per g. of total vitamin B_6, and the drip contained 5.32 μg. per ml. The percentage of total vitamin B_6 lost in the drip amounted to 8.71, which differs only about 1% from that reported for beef *(11)*. Examination of Table 2 shows that 16.56% of pyridoxine plus pyridoxamine were leached out in the drip, but that only 6.12% of the pyridoxal was lost in the drip. Upon calculating the percentage of pantothenic acid lost in the drip, it was found to be 6.95% of the total amount in the meat. The percentage in the drip from frozen beef reported earlier *(11)* amounted to about 4½ times as large a percentage as was found in this study. The amount of vitamin B_{12} lost in the drip amounted to 5.06% of the total in the meat. In the studies with beef, no data were available for vitamin B_{12}.

Amino acids. A summary of the data on the amino acid content of both the meat and drip, as well as percentage losses is given in Table 1. Amino acid values are in essential agreement with those reported by Lyman and Kuiken *(9)*. The percentage of isoleucine found in the drip amounted to 11.08%. When leucine was colculated on the percentage basis, it was found that 9.71% of the total leucine was lost in the drip. With the lysine, losses in the drip amounted to 8.60%, and there was a loss of 7.58% of the total methionine from the meat into the drip. When calculated on a percentage basis, the amount of tryptophan lost in the drip was 7.15%.

DISCUSSION

In order to determine whether the different percentages of amino acids and vitamins in drip were related to their solubility in water extracts, their solubility characteristics were compared with the percentages lost in the drip. Examination of the information fails to explain the variability in percentage losses between different amino acids and vitamins, since in a number of cases the less soluble components occurred in a greater proportion in the drip than the more soluble ones. Thus, it appears that the loss of amino acids and B vitamins into the drip is not due to simple water leaching alone. However, it is possible that leaching of the nutrients may occur in the form of more complex compounds than the amino acids and vitamins, since the analysis was based on the hydrolyzed drip, which does not account for differences between the free and bound forms.

The variation in percentage between individual nutrients would support the theory that the losses are related to the properties of the bound forms of the nutrients following freezing. It seems likely that denaturation may occur as a result of freezing and may alter the chemical structure of some constituents more than others, thereby resulting in a greater solubility and more leaching of these compounds. However, it would appear that freezing and thawing does not result in a complete breakdown to component amino acids and B vitamins, but to some intermediate breakdown products which vary in their solubility in water extracts.

Results indicate that a more constant proportion of the total amino acids in the frozen meat is lost on thawing than is true for the vitamins (Table 1). Since the amino acids are the building stones of the structural muscle proteins, any breakdown or denaturation might be expected to result in about the same proportionate amount of the amino acids as occur in the frozen meat. Whereas, the vitamins are frequently bound in enzymes and other biological compounds that differ in distribution and concentration from the structural proteins, and therefore, may be affected much differently by freezing. Evidence for the theory that the various B-vitamins may be freeze-denatured in varying amounts depending upon their combined form is found in the differential analysis for vitamin B_6. The data given in Table 2 show that only 6.12% of the pyridoxal was lost in the drip, while with pyridoxine and pyridoxamine the percentage loss was 16.56%.

One of the major problems in the interpretation of data on nutrient losses in drip is the lack of knowledge on factors influencing both volume and composition. Although Cook *et al. (3)* in 1926 reported that there was little variation in protein content between drip obtained from beef, lamb, veal or pork, methods for determining the individual components in the protein fraction were not available. Frequent mention has been made in the literature that surface area is related to the volume of drip, yet controlled studies relating surface area to volume and composition are lacking. In addition, the influence of species, individual animal variation, the influence of age and thawing conditions need further investigation before the data available can be completely evaluated.

CONCLUSIONS

The drip collected upon thawing frozen pork contained considerable amounts of the B-vitamins and amino acids. The percentage of the B-vitamins found in the drip ranged from 4.15 for riboflavin to 10.69% of the niacin. With the amino acids, the losses were from 7.15 for tryptophan to 11.08% for isoleucine. The percentage losses for amino acids were more uniform than was true for B-complex vitamins. The magnitude of the losses did not appear to be associated with the solubility in water of the individual amino acids or B-vitamins, which would indicate that the losses in drip, at least in part, are due to the leaching of more complex substances.

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EFFECTS OF CATHODE RAY AND GAMMA RAY IRRADIATION ON THE ANTHOCYANIN PIGMENTS OF STRAWBERRIES [a, b]

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Many fruits, and some vegetables, owe their attractive red or purple colors to the anthocyanin pigments which they contain. For the preparation and preservation of most products involving such fruits or vegetables, the application of heat is necessary. During such heat processing and subsequent storage, the anthocyanin pigments are partially degraded, causing a color change and concurrent reduction in the esthetic appeal of the products. The benefits of low temperature concentration on color and pigment retention in strawberry products are well established *(3, 5)*. Ionizing radiations offer a possible means of sterilizing, without heat, jellies and preserves concentrated under vacuum at low temperatures. The effects of such radiations on the anthocyanin pigments of strawberries, however, have not been thoroughly investigated heretofore.

Hannan *(4)* irradiated strawberries with 2 MeV cathode rays, in air, and noticed increased bleaching of the fruit as the dose of irradiation increased from 0.1 to 4.0 megarep (0.093 to 3.72 megarad).

In the present study, the strawberry anthocyanins were irradiated in strawberry juice, in products made of this juice, and in solutions of pure pigment isolated from strawberries. Gamma rays and 2 MeV cathode rays were used for irradiation, and their effect on the pigments was studied quantitatively.

EXPERIMENTAL

Irradiation of strawberry juice and pigment solutions. Strawberry juice, and pure strawberry anthocyanin in buffer solution were irradiated with 2 MeV cathode rays. The juice was extracted by means of a wooden hand press from frozen strawberries of mixed local varieties. The principal strawberry anthocyanin, pelargonidin-3-monoglucoside, was purified chromatographically by a method described in a previous communication *(5)*, and dissolved at a concentration of about 0.3 mM in citrate-HCl buffer solution (0.05 M disodium citrate) at pH 3.4.

Enough juice, or pigment in the buffered solution, was dispensed in 0.8 mm. O.D. Pyrex tubes so that, when the tubes were horizontal, the total thickness of upper glass wall plus sample was approximately 0.6 g/cm.[2] A 2 MeV electron acceleration [e] was used as a source for cathode rays. The tubes were irradiated horizontally in a vertical beam, either at ambient temperature, or frozen. In the latter case, the tubes rested on a bed of dry ice during irradiation.

Samples in tubes in which the air in the headspace was replaced by nitrogen were also irradiated. To prepare them, one end of the tubes, containing the samples, was drawn to

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[b] Contribution No. 1159 of the University of Massachusetts College of Agriculture Experiment Station, Amherst, Mass. This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned No. 924 in the series of papers approved for publication.

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an open capillary; the tubes were placed almost horizontally in a vacuum desiccator, and the pressure was quickly reduced to 3 cm. of mercury. Nitrogen, washed by passage through alkaline pyrogallol, was introduced into the evacuated desiccator and the evacuation and replacement procedures were repeated 5 times, before the capillary was sealed.

The anthocyanin content was determined by the method of Sondheimer and Kertesz *(12)*, and the average results of two such determinations carried out on replicate tubes are reported herein.

Samples prepared as described above were also irradiated using gamma rays.[f] Samples were packed in dry ice during shipment to the irradiation site. For irradiation in the frozen state, the samples were kept in dry ice until irradiation time. They were then placed in No. 2 cans and lowered into the source. When similar samples, kept outside the source, at room temperature, showed signs of melting, the samples being irradiated were taken out, refrozen in an acetone-dry ice mixture, and returned to the source, for completion of the specified exposure. The irradiation dosage rates were from 0.46×10^4 to 3.23×10^4 rads per minute, as measured by ferrous dosimetry.

Irradiation of dehydrated strawberry juice. Strawberry juice was dehydrated and irradiated at sub-zero temperatures. The dehydration was effected by spreading the juice in a thin film and exposing it to low pressure (3 cm. Hg) until reduced to brittle flakes. The dehydrated juice, in sealed 0.8 mm. Pyrex tubes was irradiated using gamma and cathode rays, as described in the previous section. In carrying out pigment determinations on the dried juice, the latter was first rehydrated to its original volume.

Irradiation of sweetened strawberry juice. Total solids of samples of strawberry juice was increased from 7% to 14%, 28%, and 56%, by the addition of commercial sucrose. The sugar was dissolved in the juice at room temperature, and the solids content checked refractometrically. The juices were irradiated with gamma rays, in the usual tubes, at sub-zero temperatures. However, thermos bottles, rather than cans, were used as carriers of the chilled tubes into the irradiation source.

Irradiation of strawberry jelly. Using strawberry juice and commercial sucrose 45:55 jellies were prepared by the customary method *(7)*. Jellies were concentrated to 65% solids either under atmospheric pressure (i.e. finished at 221° F.) in a glass vessel, or under reduced pressure. In the latter case, a rotating glass evaporator, connected to an aspirator, and immersed in water at 140° F., was used. The jellies were filled into 5-ounce baby food jars and capped. The conventionally-concentrated jelly and samples of the vacuum-concentrated jelly were not irradiated, while the remaining sample received two different doses of 2 MeV cathode rays, at ambient temperature. The rays hit the surface of the jelly after passing vertically through the cap, whose thickness of 0.02 g./cm^2 was well below the limit of effective penetration to these rays (0. 6 g./cm^2). One jar from each group was analyzed for pigment content immediately after preparation and irradiation, and one jar after 30 days of storage at 95° F.

Irradiation of strawberry juice containing added ascorbic acid. Samples of strawberry juice, to which 100 mg./ml. of ascorbic acid had been added, were also irradiated with gamma and cathode rays, in the frozen state. The thermos bottle technique was used in the gamma radiation. The original ascorbic acid content of the strawberry juice was found to be 35 mg/100 ml. *(11)*.

RESULTS

Results of the irradiation experiments are reported in Figures 1–6 and Table 1. Figures 1 and 2 show the effects of cathode ray and gamma ray irradiation respectively, on pigment retention in 1) strawberry juice irradiated at ambient temperature, 2) frozen, 3) under nitrogen gas at ambient temperatures or 4) frozen after dehydration. Figure 2 shows in addition the effect of gamma irradiation on nitrogen-packed frozen strawberry juice.

Figures 3 and 4 show the effects of cathode ray and gamma ray irradiation respectively on strawberry pigment in buffered citrate solutions at pH 3.4 irradiated under 1) air at ambient temperature, 2) under air, frozen, 3) under nitrogen at ambient temperature, or 4) under nitrogen, frozen. The effect of gamma irradiation on nitrogen-packed frozen pigment solution is also shown in Figure 4.

[f] At the Argonne National Laboratory, Lemont, Illinois.

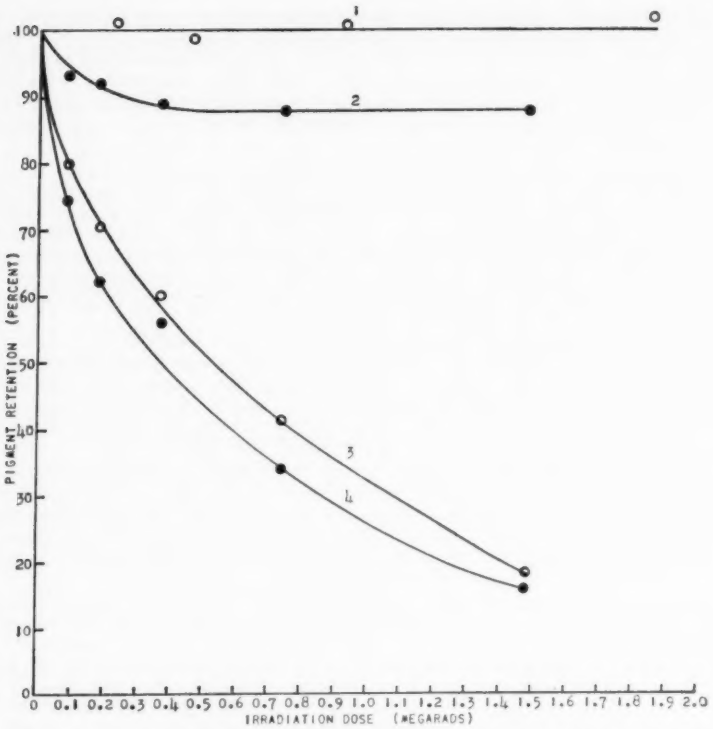

Figure 1. Effect of dehydration and of oxygen on pigment retention in strawberry juice irradiated by cathode rays. 1—Dehydrated strawberry juice irradiated at sub-freezing temperature. 2—Frozen strawberry juice. 3—Nitrogen-packed strawberry juice irradiated at ambient temperature. 4—Air packed strawberry juice irradiated at ambient temperature.

Figure 5 shows the effect of varying solids contents on pigment retention on frozen strawberry juice irradiated with gamma rays, whereas Figure 6 shows the effects of adding ascorbic acid (100 mg./100 ml.) to strawberry juice subjected to cathode ray and gamma ray irradiation.

Pigment retention in all figures shown was calculated from data obtained by analyzing the samples for pigment content, and using, as the initial pigment content, the

TABLE 1

Pigment content of unirradiated and cathode ray-irradiated strawberry jellies stored for thirty days at 95° F.

Concentration temperature (° F.)	Irradiation dose (megarads)	Pelargonidin-3-monoglucoside content (mg. %)	
		After processing	After storage
140	0	4.7	2.5
140	0.093	4.4	2.4
140	0.465	3.6	2.5
221	0	2.4	1.6

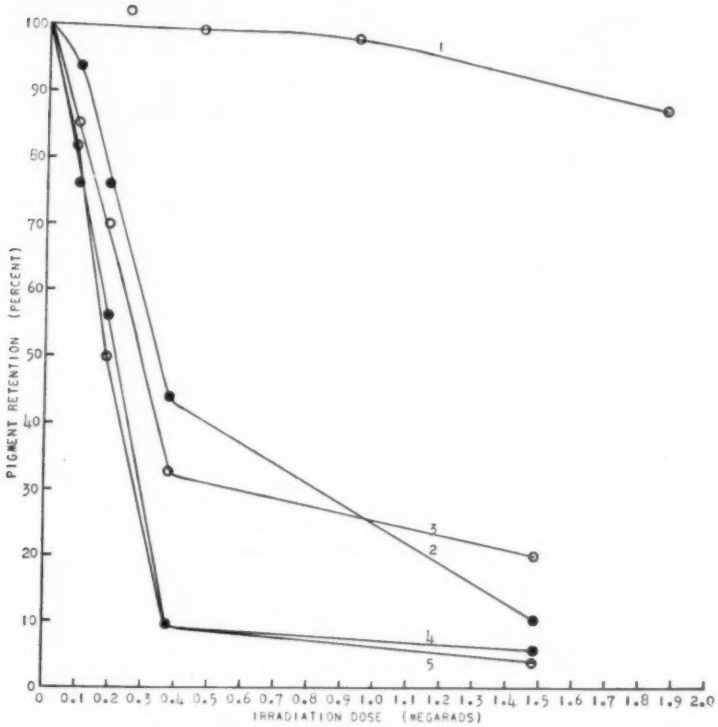

Figure 2. Effect of irradiation temperature, nitrogen headspace, and dehydration on pigment retention in strawberry juice irradiated with gamma rays. 1—Dehydrated strawberry juice irradiated at sub-freezing temperatures. 2—Nitrogen-packed frozen strawberry juice. 3—Air-packed frozen strawberry juice. 4—Nitrogen-packed strawberry juice irradiated at ambient temperature. 5—Air-packed strawberry juice irradiated at ambient temperature.

values obtained on non-irradiated control samples shipped to and from the site of irradiation along with the treated samples.

Table 1 shows the pigment contents of cathode ray irradiated jellies and control jelly samples, analyzed immediately upon irradiation, and again after 30 days storage at 95° F. It should be noted that the irradiated samples concentrated at 140° F., and those receiving a dose of 0.093 megarad, developed surface mold growth. Pigment determination on moldy samples were, of course, performed on sections of jelly drawn from well below the surface.

DISCUSSION

Results of these exploratory experiments indicate that the red strawberry anthocyanin, when isolated from strawberry juice and redissolved in citrate buffer of the same pH as that of the juice, is more sensitive to gamma or cathode rays than in its original state in the juice. This holds true whether the irradiation occurs under air or oxygen or whether the samples are irradiated at ambient temperature or in the frozen state. The protective effect of the juice

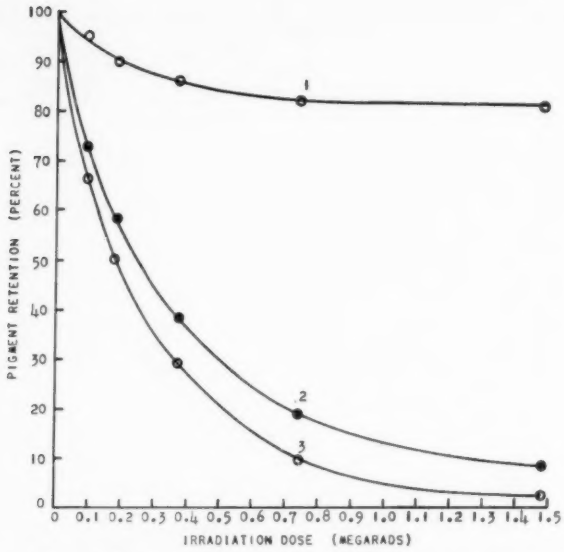

Figure 3. Effect of irradiation temperature and nitrogen headspace on retention of strawberry anthocyanin in citrate buffer at pH 3.4 irradiated with cathode rays. 1—Frozen strawberry anthocyanin solution. 2—Nitrogen-packed strawberry anthocyanin solution irradiated at ambient temperature. 3—Air-packed strawberry anthocyanin solution irradiated at ambient temperature.

could be attributed to either the presence of a specific stabilizer in the juice, or to the higher concentration of solutes in the juice.

The possibility that the radiosensitivity of the pigment is altered due to irreversible changes occurring during its isolation from the juice *(10)*, could be checked by adding back to juice isolated pigment, and comparing the rate of destruction of the pigment in this enriched juice with that in the original juice. Such a test has not been carried out, however.

Experiments using sugared and dried juices were performed in an attempt to further study the effect of increased concentration of solutes on the radiosensitivity of the pigment. Although the irradiation of dehydrated juice showed clearly that the presence of water must be involved in the destruction process of the pigment—probably through formation of free radicals originating from water—the experiment with sugared juice did not indicate any protection by solutes. Since increasing percentages of pigment were destroyed as the sugar concentration increased, it appears that the sugar molecules, upon irradiation, functioned as free radical producers, or precursors of other substances harmful to the pigment, rather than as free radical acceptors *(6)*.

The experiment using different irradiation temperatures indicated that the strawberry pigment is more radioresistant when the sample is subjected to irradiation in the frozen state than when it is in the liquid state. This can probably be explained by the immobilization of the free radicals in the frozen

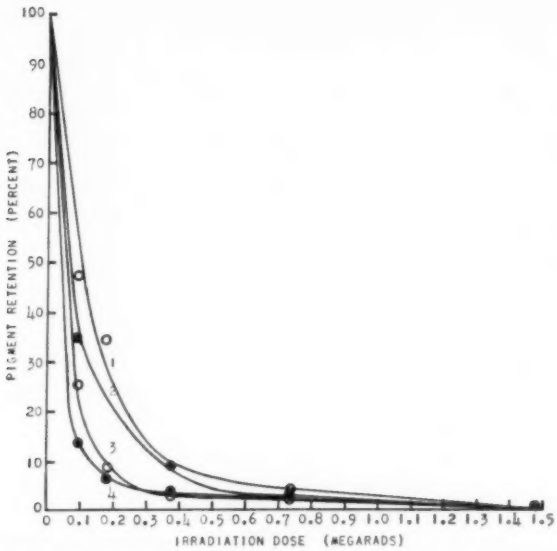

Figure 4. Effect of irradiation temperature and nitrogen headspace on retention of strawberry anthocyanin in citrate buffer at pH 3.4 irradiated by gamma rays. 1—Air-packed frozen strawberry anthocyanin solution. 2—Nitrogen-packed frozen strawberry anthocyanin solution. 3—Air-packed strawberry anthocyanin solution irradiated at ambient temperature. 4—Nitrogen-packed strawberry anthocyanin solution irradiated at ambient temperature.

sample, and is in accordance with previous experience of other investigators *(8)*.

The presence of oxygen in the sample seemed to be detrimental to the pigment when cathode rays were used for irradiation. This effect was not clearly indicated in the case of irradiation with gamma rays.

In terms of ionic yield, 0.1 (at 10^6 rad) to 0.3 (at 10^5 rad) molecules of anthocyanin are destroyed per ion pair produced by cathode rays in 0.3 mM solution of the pigment in citrate buffer (pH 3.4), the yield being slightly higher in the presence of oxygen than in the absence of it. This relatively low ionic yield may be explained by either the back reaction $H+OH \rightarrow H_2O$, which may occur in dilute solutions, or by the competition for free radicals by the buffer solutes, or by both.

The surface irradiation of jellies represented an effort to combine low temperature heat treatment, which is desirable from the color and flavor standpoints, with prevention of mold growth, which is the most likely kind of microbial spoilage for underprocessed high solids fruit spreads. The fact that mold growth did not appear, after storage, in the low temperature concentrated jelly which received 0.465 megarads of cathode rays, while it did appear in the other samples of low temperature concentrated jelly, does not definitely prove that this dose is adequate for surface "sterilization" of such jelly, since the number of samples used was too small for drawing such a conclusion. The

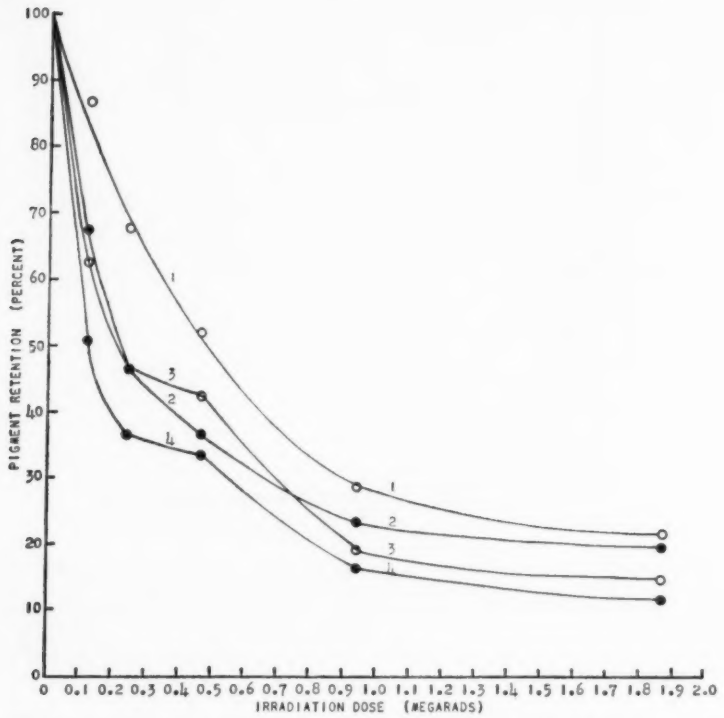

Figure 5. Effect of total solids content on anthocyanin retention in frozen strawberry juice irradiated by gamma rays. 1—7% Total solids (no added sucrose). 2—14% Total solids (7% sucrose added). 3—28% Total solids (21% sucrose added). 4—56% Total solids (49% sucrose added).

data show clearly, however, that preparation of jelly at low temperature, followed by surface irradiation results in much higher pigment retention, both immediately after irradiation and after storage at spoilage temperatures, than occurred in jelly prepared by the open-kettle method. It should be noted that no off-flavors were detectable in the irradiated jellies.

Ascorbic acid has been suggested as a protective compound against side effects of food irradiation *(9)*, although it was not able to protect the color of meat in irradiation *(1)*. In the case of the strawberry pigment, no protective effect of the ascorbic acid could be demonstrated either.

It should be pointed out that in practically all instances in which a comparison was made of the effect of cathode and gamma rays on the red strawberry pigment, equivalent doses of cathode rays were found less destructive to the pigment than gamma rays. Such an effect could be connected with the different dose rates of the two kinds of radiation used, slower dose rates appearing to be more detrimental to the pigment. In agreement with this are the findings of other investigators on dyes in solution which were less bleached by

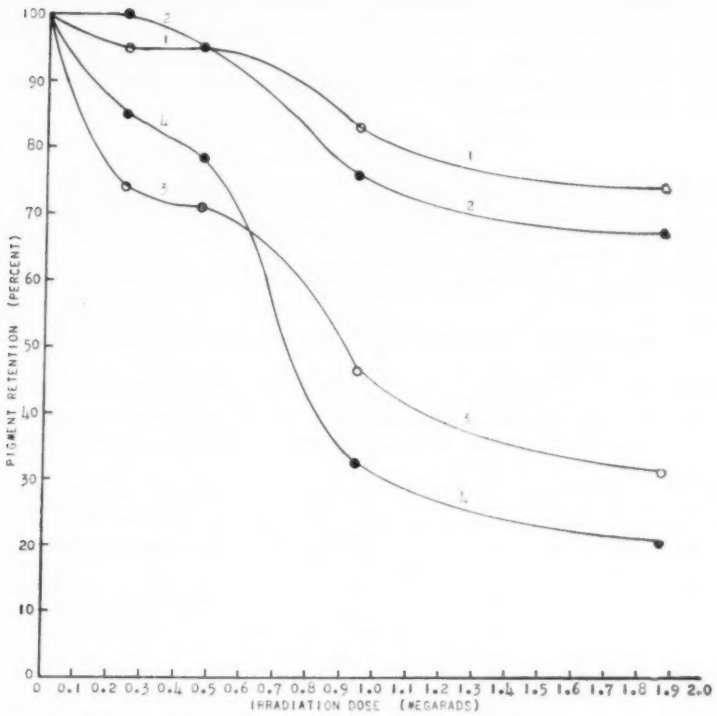

Figure 6. Effect of ascorbic acid in pigment retention in strawberry juice irradiated with cathode or gamma rays. 1—Cathode ray-irradiated strawberry juice containing 100 mg./100 ml. added ascorbic acid. 2—Cathode ray-irradiated strawberry juice control. 3—Gamma ray-irradiated strawberry juice control. 4—Gamma ray-irradiated strawberry juice containing 100 mg./100 ml. added ascorbic acid.

one large pulse of cathode rays than by a number of small pulses, and this bleaching in turn was less than that which occurred with weaker continuous gamma radiation *(2)*, all of the same total irradiation dose.

SUMMARY

The strawberry anthocyanin pigment is generally sensitive to ionizing radiations, 0.465 megarads of 2 MeV cathode rays destroying 55% of the pigment in strawberry juice, and the same dose of gamma rays destroying 63% of the pigment.

Irradiation of frozen juice results in reduced pigment destruction and dehydration of the juice further increases the radioresistance of the pigment. The strawberry anthocyanin is more radiosensitive in pure pigment solutions than in the original juice. Addition of sucrose to juice increases the pigment destruction by irradiation with gamma rays.

Combination of low temperature preparation of strawberry jelly with surface irradiation results in higher pigment retention than in conventional open-kettle jelly cooking.

Ascorbic acid does not seem to prevent the detrimental effect of irradiation on the strawberry anthocyanin.

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LYSOZYME STUDIES ON CHICKEN EGG CHALAZAE[a]

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Lysozyme, an important protein in the egg, is believed to be associated in some way with thinning of the firm albumen. It will lyse (destroy the cell wall) many bacteria. Without lysozyme, the egg would be much more perishable than it is. Considering the importance of lysozyme, the relation between lysozyme and chalazae, rather infrequently studied, merits further investigation.

Many investigators have worked with egg albumen, excluding the chalazae as a source of lysozyme. Wilcox and Cole *(14)* have shown that wet egg albumen contains approximately 4.5 to 5 mg. of lysozyme per milliliter. Cotterill and Winter *(4)* found that the lysozyme content of egg albumen varied from about 3% to 5% based on liquid white; 3% to 4% based on total solids and 3.5% to 4.7% based on total protein. All of these investigators state that there is very little difference in the lysozyme content of the thick and thin albumen. Smolelis and Hartsell *(10)* reported that the lysozyme content of chicken egg albumen, on a wet basis, is approximately 6.5 mg. per ml. as compared to 6.9 for the bantam, 6.7 for quail, 3.4 for pheasant, 3.1 for the guinea hen and 1.8 for duck egg albumen.

In most of the work done on lysozyme content of eggs, the investigators have been careful to pick all of the chalazae material out of the rest of the albumen. Sugihara *et al. (13)*, however, reported that the lysozyme content of chalazae cords in chicken eggs is two to three times greater than the rest of the albumen. Since their study concerned itself with virus antihemagglutinin activities of avian egg components, very little information was given on how they arrived at this figure.

Lysozyme action and assay methods. Since the discovery of lysozyme by Fleming *(5)* many methods have been used to determine its activity. Preparations containing the enzyme have been assayed according to the criterion of lysis *(3, 5, 6, 9, 11, 14)*.

Similarly, assay methods have been perfected which utilized growth inhibition *(3, 5, 6, 9, 11, 14)* or viscosimetry *(8)*. Currently, assay techniques which reveal gradients in the clearing of a suspension of a sensitive bacterium, usually *Micrococcus lysodeikticus*, as the concentration of enzyme varies, are most frequently employed. The sensitivity, the quantitative aspects, and the relative simplicity of the Smolelis and Hartsell technique *(11)*, as further developed and applied *(1, 10)* offered a procedure which was simple and convenient. Modifications suggested by Wilcox and Cole *(14)* and Wilcox and Daniel *(15)* point to the complexing of lysozyme and the influence of the sodium ion on lysis. In this study, the basic cultural preparations, having reproducible and consistent sensitivity to lysozyme, as well as the physical

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conditions, especially temperature for lysis, were significant factors in selecting this basic technique.

EXPERIMENTAL PROCEDURE

The method used for the lysozyme assay in this study was primarily that of Smolelis and Hartsell *(11)*. Some modifications in the pH and dibasic sodium phosphate of Sorensen's phosphate buffer were made as the study progressed. This assay method is based on a comparison of light transmission of crystalline lysozyme dilutions with the values for the substance being tested after the addition of lysozyme susceptible cells and incubation. A crystalline lysozyme secured from Armour and Company was used for the standard. The preparation of *Micrococcus lysodeikticus* was obtained from Difco Laboratories, Detroit, Michigan, as a powder ready for rehydration. These cells were killed by the Smolelis-Hartsell method *(11)* using ultraviolet light.

The chalazae cords used for most of this study were taken from eggs of one strain of Single Comb White Leghorns at the Purdue University Poultry farm. Fourteen strains of birds from the North Central Regional Laboratory were also used for part of the experiment.

In all phases of this study, one-day-old eggs were used. They were broken-out and the chalazae were pulled from the thick albumen with tweezers. As much thick albumen as possible was removed from the chalazae cords with small shears. The trimmed cords were then put in distilled water for approximately 30 minutes. After soaking, the cords were placed in a fine mesh strainer and washed with distilled water 4 separate times. The washed chalazae were then blotted with paper towels to remove excess water and weighed. The cords were then placed in a vacuum oven for drying at a temperature of 40° C. under an atmospheric pressure of 25 inches of mercury. At least 20 hours were allowed to completely dry the cords. Thick and thin albumen similarly desicated were also used for part of the study.

Following drying, the chalazae cords and other albumen when used, were ground into a fine powder with a mortar and pestle. The fine material, to give a final dilution of 1:100, was then rehydrated in distilled water for 30 minutes. The rehydrated material was further broken up into a fine suspension with a Waring blender operating for one minute. Regulation to 40 volts prevented excessive foaming of the preparation.

Prior to assay, rehydration of *Micrococcus lysodeikticus* in the buffer provided a cell suspension which was then adjusted to 10% transmission in a Coleman spectrophotometer, Model 14, equipped with a PC-4 filter at a wave length of 540 mμ. A distilled water blank at 100% transmission was the control.

A 1:1000 stock solution of crystalline lysozyme was prepared in distilled water. From this concentration, starting with 1:100,000 and progressing to 1:6,400,000 a range of 0.15 mcg. to 10 mcg. for each milliliter of dilution was made. With the transmission values for the crystalline lysozyme mixtures, standard curves were prepared by plotting optical density against concentration (data in Table 1). A log scale was used for concentrations

TABLE 1

Standard curves involving different pH and sodium phosphate levels [1]

Dilutions	pH 6.2 Na_2HPO_4 9.47 g. % T	pH 6.2 Na_2HPO_4 23.07 g. % T	pH 7.2 Na_2HPO_4 9.47 g. % T	pH 7.2 Na_2HPO_4 23.07 g. % T
1:100,000	84.0	88.0	90.5	83.5
1:200,000	76.5	82.0	86.5	76.5
1:400,000	61.0	65.0	74.5	61.5
1:800,000	44.5	47.0	54.5	43.5
1:1,600,000	34.5	35.0	39.0	33.0
1:3,200,000	27.0	29.0	31.0	27.0
1:6,400,000	24.5	25.5	24.0	25.0
Control	23.0	23.0	23.0	23.0

[1] Room Temperature (25° C. approximately).

on the abscissa of the standard curve. The optical density values are located on the ordinate.

Matched tubes showing the same lignt transmission with distilled water received 3 ml. of the lysozyme dilution and 3 ml. of the cell suspension. All mixtures were made in duplicate and 3 dilutions were made for each unknown. Trial runs were made so that it was possible to have all 3 dilutions fall on the straight line part of the standard curve.

To obtain the concentration of lysozyme of the unknowns, the optical density values were located on the ordinate and then projected to the standard curve. By projecting to the abscissa from these points, the concentration of lysozyme in each dilution was determined. Multiplication by the dilution factor gave the concentration of lysozyme per milliliter of undiluted dry material.

Preparation of chalazae. Since very little work has ever been done on determining the lysozyme content of chalazae cords, much of the study in the first experiments were carried out to determine techniques for preparing chalazae for assaying. Sugihara *et al. (13)* removed the chalazae from the thick white with tweezers and then stored them in a buffered saline solution for several hours. Each chalaza was freed from any extraneous thick albumen and homogenized in a thioglycol-buffer mixture by means of a ground-glass hand homogenizer.

A modified Sugihara method was developed which gave quite uniform results. This method is explained earlier in this paper. It was noted that when the chalazae were compact, the job of removing extraneous albumen was fairly easy. When the cords were stringy, however, it was extremely difficult to remove the adhering albumen. This difficulty may have accounted for some of the variation found in this study.

EXPERIMENTAL AND RESULTS

Technique studies. Certain procedures and methods were thought to influence the lysozyme content of the chalazae; therefore, the following studies were made with results as reported. Particle size was first considered since maximum solubilization of the enzyme was desired.

Particle size: For reducing the size of rehydrated chalazae particles, a Waring blender alone was compared with a Waring blender plus a hand homogenizer. Homogenization, apparently, had an additional effect on reducing the size of the chalazae particle. With the Waring blender alone, the lysozyme content of the cords was 86 mg. per g., while with the Waring blender plus the homogenizer, only 74 mg. per g. resulted. It seems evident from this that homogenization denatured some of the lysozyme, or if released, it was immediately complexed with other compounds and could not be detected.

Filtration: It seemed possible that the removal of some of the larger particles might increase the rate of light transmission when using the turbidemetric method of assay. The opposite is true, however, because with the filtered material only 70 mg. of lysozyme per g. of dried chalazae resulted; with the non-filtered material the lysozyme content was 78 mg. per g. Evidently the particles which were filtered out contained a considerable amount of lysozyme.

H-ion and buffer: As described earlier in this paper, there is some controversy in the literature concerning the effect of pH on the lytic activity when using the turbidemetric method for assaying. Wilcox and Daniel *(15)* claimed that the concentration of sodium ion had to be optimal for the lytic activity of lysozyme. For this reason it was decided to vary the sodium ion concentration of Sorensen's buffer to discern the effect on the assay. Four standard curves were first made, by using two different pH levels and two different concentrations of hydrated di-basic sodium phosphate.[c]

These data seem to point out that a pH of 7.2 with only 9.47 g. of dibasic sodium phosphate in the buffer give the highest readings of light transmission using pure crystalline lysozyme. Phosphate buffer pH 6.2 containing 23.07 g. of dibasic sodium phosphate per liter, which is recommended by Smolelis and Hartsell *(11)*, also give a high light transmission. At pH of 6.2, the higher concentration of dibasic sodium phosphate

[c] The hydrated dibasic sodium phosphate used contained 12 molecular weights of water for each molecular weight of dibasic sodium phosphate.

allows the highest light transmission, therefore the greatest clearing, while with a pH of 7.2, the lower concentration of sodium gives the highest transmission. It is possible that there is an interrelationship between pH and sodium ion concentration. The phases of adsorption, depolymerization and solubilization in lysozyme lysis are known to be influenced by pH and by the cation.

To carry this study further, a range of pH values were used: 5.2, 5.8, 6.2, 6.5, 7.2, 7.5 and 8.0. Each of the different pH levels was prepared from stock solution, respectively, of dibasic sodium phosphate containing 9.47 g. per liter and potassium dihydrogen phosphate having 9.08 g. per liter. Results are listed in Table 2.

This information points out that of the various pH levels used, 7.2 allowed for the greatest lysis when pure lysozyme was used at room temperature. This could be interpreted to mean that the reacting polar groups of the pure crystalline lysozyme molecule are more completely ionized at pH of 7.2 than at the other pH levels used, therefore adsorption is facilitated, or that the solubilization of *Micrococcus lysodeikticus* cells is of a different magnitude at the different pH levels. According to Grula and Hartsell *(7)* possible effects from autolytic factors must be assessed with caution.

The same pH levels were used when assaying rehydrated chalazae to find out if the results were similar (Table 3). The dibasic sodium phosphate content of the buffer was again 9.47 g. per liter used in the stock solution.

TABLE 2

Effect of pH of buffer on per cent light transmission; pure lysozyme (1:200,000 dilution)

pH	% T	pH	% T
5.2	55.0	7.2	87.0
5.8	70.0	7.5	84.0
6.2	76.5	8.0	82.0
6.5	78.5		

TABLE 3

Effect of pH of buffer on per cent light transmission: dried chalazae (1:40,000 dilution)

pH	% T	pH	% T
5.2	36.0	7.2	68.5
5.8	44.5	7.5	68.5
6.2	48.0	8.0	66.5
6.5	48.0		

Although the results are not as striking as was the case with pure lysozyme, it appears that a pH of about 7.2 for the buffer is associated with the highest per cent light transmission. From these data one would conclude that for the turbidemetric method of assaying for lysozyme, a buffer with pH of 7.2 should be satisfactory at room temperature. Since this study showed that the buffer at pH 7.2 containing 9.47 g. of dibasic sodium phosphate per liter of stock solution gave the highest per cent light transmission at room temperature, this combination was used for the remainder of the study.

Lysozyme content of dried chalazae. A comparison was made of dried chalazae cords from a Purdue Strain of 100 Single Comb White Leghorns. The study represents eggs from 6 different days produced at weekly intervals. The results (Table 4) give both the milligrams of lysozyme per gram of dried chalazae and the percentage of lysozyme of the total protein of the cords.

These results, except for trial 2, indicate relatively good agreement among replications of the assy technique. Values above 90 mg. contrast sharply with those obtained with

TABLE 4

Lysozyme content of chalazae of one strain of chickens

Trial No.	Lysozyme concentration mg./g.	% Lysozyme of protein [1]
1	93	11.67
2	72	9.04
3	94	11.80
4	93	11.67
5	95	11.92
6	92	11.54

[1] Protein determinations were made by using a micro-Kjeldahl apparatus and then converting nitrogen content to protein with a conversion factor of 6.25.

albumen by other investigators. A possible explanation for trial 2 being low, is that more extraneous albumen was left on the cords that particular day.

Fourteen strains of chickens from the North Central Regional Laboratory were used in a study to compare the lysozyme content of the chalazae. Fifteen eggs were broken-out from each strain and the chalazae were processed as previously described.

These strains show (Table 5) a tremendous difference in lysozyme content of the chalazae. Some of this variation might be explained by technique, since with some of the strains the cords were much more difficult to clear of extraneous material than others. It is not surprising that these strains varied in their lysozyme content of the chalazae. Cotterill and Winter *(4)* have shown that the lysozyme content of the albumen varies among strains.

Analysis of variance confirmed the very high significance of the differences among strains (Table 6).

Effect of enzymes and incubation on the lysozyme activity of the chalazae. Some investigators believe that the gel-like consistency of the thick albumen of an egg is due to a complexing of 2 proteins, lysozyme and ovomucin. They feel that when

TABLE 5

Lysozyme content of chalazae of fourteen strains of chickens [1]

Strain No.	Lysozyme Content mg./g.	Strain No.	Lysozyme Content mg./g.
125	108	119	76
122	92	128	72
122	83	205	72
206	82	121	60
139	80	105	45
134	79	143	45
112	76	209	44

[1] Based on Tukey's test as described by Snedecor *(15)*, significant differences were found between strains as separated by the solid lines.

TABLE 6

Analysis of variance of Table 5 (fourteen strains)

Source of Variation	d.f.	Sum of squares	Mean value	F value
Total	27	8965		
Strains	13	8891	683.92	129.29 [1]
Error	14	74	5.92	

[1] Significant at the 1 per cent level.

this complex breaks due to a change in the pH, thinning of the albumen takes place. Since the chalazae cords are thought to be merely dense albumen, one would assume that the same complexing between lysozyme and ovomucin would occur. If this is true, then anything that would break the complex between these 2 proteins should result in greater lysozyme activity, and thus give a more accurate assay of the actual lysozyme content of the chalazae. The ideal way would be to split ovomucin and release lysozyme without breaking up the lysozyme molecule.

It appeared that the logical approach was to use a mucinase, an enzyme which is specifically used for attacking ovomucin. Bergamini *(2)* states that several bacteria including *Proteus vulgaris, pseudomonas pyocyanea, Serratia marcescens, Staphlococcus aureus, Micrococcus freudenreichii* and *Streptococcus liquefaciens* produce a mucinase. Supernatents from these bacteria were used in the study.

The bacteria were grown in tryptone proteose peptone broth containing 25 g. of tryptone and 10 g. of proteose peptone per 1000 ml. for 24 hours. The cells in the media were then centrifuged at 3000 revolutions per minute, for 20 minutes. Dilutions of 1:10 and 1:100 of the supernatent were made with buffer. These 2 dilutions were then mixed with an equal proportion of a 1:100 chalazae suspension, thus giving a "mucinase" concentration of 1:20 and 1:200. The "mucinase" and chalazae were incubated at 37° C. for 48 hours and then assayed by the regular procedure. The results can be found in Table 7.

There was very little difference in the lysozyme content between the various bacteria used and the control with 1:20 dilution of "mucinase." A possible explanation is that the "mucinase" if present, was in such high concentration that it broke down the lysozyme molecule as well as the ovomucin. There was, however, quite a variation in lysozyme content of the same dry chalazae material when a 1:200 concentration of "mucinase" was used. Analysis of variance was used to see if these differences were significant (Table 8).

TABLE 7

Effect of bacterial mucinase on lysozyme content of chalazae

Bacteria used	1:20 Concentration mg./g.	1:200 Concentration mg./g.
Proteus vulgaris	83	100
Pseudomonas pyocyanea	83	80
Serratia marcescens	81	88
Staphlococcus aureus	74	72
Micrococcus freudenreichii	77	80
Streptococcus liquefaciens	76	74
No "mucinase"	76	76

TABLE 8

Analysis of variance of Table 7 (1:200 dilution of bacterial "mucinase")

Source of variation	d.f.	Sum of squares	Mean square	F value
Total	13	1169		
Treatments	6	1131	188.5	34.7 [1]
Error	7	38	5.43	

[1] Significant at the 1 per cent level.

With the 1:200 dilution of "mucinase" the analysis of variance test shows that there is a difference among the treatments which is highly significant. Tukey's test was used to see where the difference occurred. The results show that *Proteus vulgaris* and *Serratis marcescens* are significantly different from the control where no "mucinase" was used. These results are interesting and important. If "mucinase" can be used to separate lysozyme from ovomucin, it means that a more accurate measure of the actual amount of lysozyme in chalaza cords or egg albumen is available. Implications involving the

association between egg spoilage and the production of a "mucinase" by microbes are interesting to project.

Another study was set up to see if incubation of the chalazae material in distilled water for 24 hours would have any effect on the lytic activity. Chalazae material was suspended in distilled water as described before. In one case, the 1:100 suspension was allowed to incubate at 37° C. for 24 hours while the control was assayed within one hour after the suspension was made. The results show that incubation at 37° C. does result in a higher lysozyme content. With the same chalazae material, the incubated chalazae shows a concentration of 136 mg. per gram while those not incubated have a concentration of 96. This could mean that when lysozyme separates from ovomucin, just by standing at 37° C. for 24 hours or more, complete rehydration takes place.

Comparison of lysozyme content of chalazae cords and thick and thin albumen. The final phase of the lysozyme study was to compare the lysozyme content of chalazae with the rest of the albumen of eggs. This was done at two pH levels, namely; 6.2 and 7.2. Two concentrations of dibasic sodium phosphate were used, namely; 9.47 and 23.07 g. per liter. The object of the different levels of pH and dibasic sodium phosphate was to find if the thick and thin albumen responded similarly to the chalazae in respect to pH and sodium ion concentration as noted above. Results are given in Table 9.

Considerable information was obtained from this particular study. These data show that the chalazae cords contains approximately two to three times as much lysozyme as the rest of the albumen. This is an agreement with Sugihara *et. al. (13)*. Where the concentration of dibasic sodium phosphate is 23.07 g. per liter as in Sorensen's buffer, the concentration of lysozyme in the thick and thin albumen is very similar. This agrees with the work of Wilcox and Cole *(14)* and Cotterill and Winter *(4)*. With a dibasic sodium phosphate concentration of 9.47 g. per liter of stock solution, the lysozyme activity is lower in all cases but much lower with the thin albumen. The reason for the lysozyme content being much lower for the thin albumen as compared to the thick albumen and chalazae cords is not understood.

From this study one would conclude that a pH of 7.2 allowed for more lytic activity of the lysozyme than did a pH of 6.2 with all 3 types of albumen. In all cases the higher concentration of dibasic sodium phosphate results in an assay which gives a higher concentration of lysozyme. The lower level of dibasic sodium phosphate shows a higher transmission of light when the unknown is tested, but when compared to the standard curve, a lower concentration of lysozyme results than is the case with the higher concentration of sodium ion.

TABLE 9

Lysozyme content of the chalazae and thick and thin albumen

Source of lysozyme	pH	Na_2HPO_4 Concentration g./liter	mg./g.	% Lysozyme of protein
Chalazae	6.2	9.47	76	9.42
Chalazae	6.2	23.07	100	12.55
Chalazae	7.2	9.47	112	14.05
Chalazae	7.2	23.07	130	16.31
Thick Albumen	6.2	9.47	36	4.76
Thick Albumen	6.2	23.07	48	6.30
Thick Albumen	7.2	9.47	40	5.25
Thick Albumen	7.2	23.07	49	6.43
Thin Albumen	6.2	9.47	25	3.28
Thin Albumen	6.2	23.07	49	6.48
Thin Albumen	7.2	9.47	28	3.68
Thin Albumen	7.2	23.07	42	5.51

DISCUSSION

A major part of this study involved working out the details of a satisfactory technique for the preparation of chalazae cords for determining their lysozyme content by the turbidity method of assay. It is entirely

possible that this method of preparation could be improved by further trials and study.

It is important that the chalazae be trimmed of extraneous albumen and then soaked and washed in distilled water. The soaking and washing of the cords in distilled water apparently removes a great deal of the clinging albumen. Evidence of this is the higher lytic activity that was received in this study when the chalazae were soaked and washed in distilled water.

Drying of the chalazae cords was found to be very beneficial. First of all, when working with dry material, one does not have to be concerned with variation in water content that could easily occur among trials. More uniform results can be obtained by using dry chalazae. The dry chalazae cords can be fairly easily rehydrated and suspended in distilled water. It is very difficult on the other hand to make a fine suspension with wet chalazae material.

It was discovered in this study that a Waring blender operating with 40 volts of electricity for one minute does a good job of forming a fine suspension when rehydrated chalazae material is used. Foaming results if the voltage is not reduced and appears to cause some inactivation of the lysozyme. Homogenizers will break up the chalazae material into a fine suspension, but it appears that the homogenization also alters lysozyme somewhat.

The turbidemetric method of assay for lysozyme activity as described by Smolelis and Hartsell *(11)* seemed to be a satisfactory method for determining lytic activity of the chalazae. It was discovered, however, that using Sorensen's phosphate buffer at a pH of 7.2 resulted in higher lytic readings and thus a more accurate measure of the lysozyme content of the chalazae at room temperature. Wilcox and Cole *(14)* also found that this higher pH was more satisfactory with their work on the lysozyme content of egg albumen at 10° C.

During this study on the lysozyme content of chalazae, it was felt that a buffer with the lower concentration of dibasic sodium phosphate namely; 9.47 g. per liter of stock solution and a pH of 7.2 was the most satisfactory. With 9.47 g. of dibasic sodium phosphate, the per cent of transmission was greater with both the unknown and the pure crystalline lysozyme. In summarizing the data, however, and comparing the unknown to the standard curve, it was found that the higher concentration of 23.07 g. of dibasic sodium phosphate resulted in a higher concentration of lysozyme per gram of chalazae material.

The lysozyme content of the chalazae cords used in this experiment proved to be between two and three times higher than the thin or thick albumen. This is important because it could mean that the chalazae play an important role in protecting the yolk against certain microbes. The thick white of an egg has been considered the protective mechanism of the yolk because of its lysozyme content.

Strains of chickens appear to differ in the lysozyme concentration of their chalazae. This could have a practical application if the chalazae aid in the protection of the yolk of eggs. This may be at least a partial answer as to why some strains of chickens produce eggs that hold up longer than others in the channels of trade.

An interesting part of this research was the effect of "mucinase" on the lytic activity of the chalazae cords. When *Proteus vulgaris* and *Serratis marcescens* were used for the production of "mucinase," a highly significant, different concentration of lysozyme resulted as compared to the control. Further investigation should be made on this phase of work before accepting these data. The lysozyme concentration of the control is lower than is the case in most of the rest of the study. A possible explanation for this might be the long incubation period of 48 hours at 37° C. It is possible that at this length of incubation, the lysozyme molecule is broken down. An incubation period of 24 hours at 37° C., however, proved to be beneficial and actually increased the available lysozyme content of the chalazae.

SUMMARY

A satisfactory method for the preparation of chalazae cords when determining their lysozyme content by the turbidity method of assay is reported. The turbidemetric method of assay for lysozyme as described by Smolelis and Hartsell *(11)* is satisfactory for use with chalazae. Sorensen's phosphate buffer at pH 7.2 at room temperature, however, was found to allow greater lysozyme activity than the same buffer used at pH 6.2.

This study showed that the lysozyme content of the chalazae cords was two to three times higher than the rest of the albumen. It was found that with the strains of chickens used in this study there were differences in lysozyme content of their chalazae which were highly significant.

Proteus vulgaris and *Serratia marcescens* appear to produce a "mucinase" which increases the lytic activity of the chalazae when subjected to broth cultures of these bacteria.

Allowing rehydrated chalazae material suspended in distilled water to incubate at 37° C. for 24 hours appeared to increase the available lysozyme content of the cords.

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LIPOXIDASE ACTIVITY AND OFF-FLAVOR IN UNDERBLANCHED FROZEN CORN-ON-THE-COB [a, b]

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A progressive deterioration in quality is known to occur when raw or underblanched vegetables are held in frozen storage for extended periods of time. These changes in quality, characterized mainly by the development of off-flavors, and believed to be the result of enzyme action, are described in a review by Joslyn *(9)*. Various enzymes have been suggested as causative agents in off-flavor production in frozen foods, but attention has been centered around catalase and peroxidase.

Comparative studies on extent of catalase and peroxidase inactivation and formation of off-flavors indicated that activity of these enzymes could be used as indices of the extent of heat inactivation of enzymes actually responsible for off-flavor formation. The use of these two enzymes in the control of blanching treatment and as an index of adequacy of blanching was due primarily to the relative ease with which their activity could be determined *(5, 13)*.

Testing for adequacy of blanching in corn-on-the-cob has presented several problems not normally encountered with other vegetables *(2, 14, 15)*. Tests for catalase and peroxidase are not always reliable as criteria of adequate blanching for frozen corn-on-the-cob *(3, 4, 5, 15)*. This study was undertaken to determine whether or not assays for enzymes other than catalase and peroxidase might better serve this purpose, and show a better correlation with off-flavor in the frozen product.

Recently the enzymes lipoxidase and lipase have been proposed as causative agents in the development of off-flavors, peroxidation of lipid matter, and chlorophyll losses in raw frozen peas *(20, 21, 22)*. Lipoxidase activity has been demonstrated in corn by Franke and Frehse *(7)* and by Fritz and Beevers *(8)*.

Residual lipoxidase activity and the extent of peroxidation of lipid matter were determined in commercial pack samples of frozen corn-on-the-cob, and lipoxidase activity was correlated with flavor as determined by taste tests. The effects of processing conditions on residual lipoxidase content and flavor of the frozen product were investigated.

EXPERIMENTAL

Preparation of samples. Representative samples of commercial pack frozen corn-on-the-cob of two varieties, Golden Cross and Victory Golden, were taken at regular intervals during the processing season. All of the corn had been blanched in flowing steam for 10 minutes *(2, 23)*. The corn was packaged in waxed cardboard boxes with

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a sealed overwrap of waxed paper, frozen, and held in storage at —17.8° C. The samples were removed for taste tests and prepared for chemical analysis after 6 months of frozen storage. Taste tests were run on one-half of an ear, the chemical analyses on the other half.

The samples as prepared consisted of 3 portions: *(1)* a kernel fraction (designated K series) which consisted of kernels cut down to, but excluding the major portion of the germ; *(2)* an intermediate fraction (designated C series) which consisted of the bases of the kernels including the germ and also including a layer of the cob about ⅛ inch in thickness; and *(3)* the remainder of the cob (designated COB series). All samples were stored at —17.8° C. until used, and all manipulations prior to analysis were carried out at this temperature.

Taste tests and qualitative tests for catalase and peroxidase were made on representative ears from each lot of corn after 6 months of frozen storage *(5, 13)*.

For the determination of lipoxidase activity and of peroxide number of the lipid matter, the ears were pooled in the following manner. Samples from 2 ears of corn were used in the analyses of the K series; the analyses on the C series and COB series were made on composites of 4 ears. The cobs were cut in half longitudinally with a band saw while in the frozen state. In each series the composite samples were thoroughly mixed and then divided into two equal portions, one of which was kept at —17.8° C. until analyzed, the other of which was placed at room temperature (21° C., 45% relative humidity) and held under these conditions for 5 hours prior to analysis.

For the determination of lipoxidase activity samples of 1.5 g. were removed from both the frozen samples and from those held for 5 hours at room temperature. The thawed materials were then rapidly refrozen at —17.8° C. For the determination of peroxide and acid numbers the frozen samples were lyophilized in a Stokes Freeze-Dryer and then held in friction-lid glass jars at —17.8° C. until analyzed.

Analytical methods. Lipoxidase activity was determined manometrically at 30° C., by the method of Süllmann *(17)*. In the K series 1.5 g. of material was ground with 2.5 ml. distilled water in a small Potter-Elvehjem homogenizer. In the C series 1.5 g. of material was ground as above with 4.0 ml. distilled water. In the COB series 1.5 g. of material was ground with 0.75 g. Berkshire sand and 4.0 ml. distilled water in a glass mortar. In the K and C series the ground material was pipetted into the Warburg flasks with a wide-mouth pipette; in the COB series 1.0 g. samples of the ground material were weighed directly into the Warburg flasks using a torsion balance.

The main compartment of the Warburg flask contained 1.0 ml. of tissue extract, 1.0 ml. of 0.2 M Na_2HPO_4 buffer (adjusted to pH 6.8 with 85% H_3PO_4), plus sufficient distilled water to make a final volume in the flask of 3.2 ml. The substrate, 1.0 ml. of 0.05 M linoleic acid dispersed in water, was placed in the side-arm of the flask. The center well of the flask contained 0.2 ml. of 20% KOH and a filter paper wick to absorb CO_2. Control flasks containing tissue extract, but with 1.0 ml. distilled water in place of the substrate were included in all determinations of lipoxidase activity. Blank determinations of autoxidation of the substrate were also included with every run. Readings of oxygen uptake were made at 10 minute intervals up to 60 minutes after mixing the enzyme and substrate. All values of oxygen uptake reported have been corrected for uptake without added substrate and for autoxidation of substrate.

Lyophilized samples of kernel and intermediate layer tissue were ground to a fine powder in a glass mortar. The dried cob tissue was ground in a laboratory feed mill. The samples of finely ground corn tissue were placed in paper extraction thimbles, covered with a plug of Pyrex glass wool, and extracted with anhydrous peroxide-free ethyl ether for 24 hours in all-glass Soxhlet extractors. Following removal of ether under reduced pressure, peroxide number and acid number determinations were run on 20 to 30 mg. samples of the lipid matter by the micromethods described by Lee and Wagenknecht *(10, 11)*. All analyses were run in duplicate and average values are reported.

RESULTS

Samples of commercial pack corn-on-the-cob were subjected to taste tests and to qualitative tests for catalase and peroxidase after 6 months of frozen storage at —17.8° C. All samples of Golden Cross were rated *good* in the taste tests, whereas the samples of

Victory Golden were rated *fair* to *poor*. Positive tests for catalase activity were found in the kernel and intermediate layers (K and C series) of both varieties, and positive peroxidase tests were found in the kernel, intermediate layer and cobs (K, C, and COB series) of both varieties. There was an increasing gradient of residual peroxidase activity, being lowest in the kernels and highest in the cobs. There was, however, no correlation between the amounts of residual catalase and peroxidase activity and the presence of off-flavors in certain of the samples, which had resulted in their being scored down for flavor in the taste tests.

These samples of corn showed the presence of residual lipoxidase activity (Table 1). This activity was most pronounced in the intermediate layer samples (C series), and was present also in the COB series. There was a striking varietal difference in the amount of residual lipoxidase activity. Both the intermediate layer and the cobs of the Victory Golden ears showed much higher lipoxidase activity than did the samples from the corresponding regions of the Golden Cross ears. The kernels (K series) of both varieties showed only very small amounts of residual lipoxidase activity, or none at all.

Holding the material at room temperature for 5 hours served to diminish the residual lipoxidase activity of the samples from all 3 regions of the ear. The greatest proportional reduction of activity due to holding at room temperature was apparent in samples from the intermediate layer (C series). Residual activity of the COB series was least affected by the holding treatment.

The distribution of lipoxidase activity within the raw corn ear was determined on samples of raw corn stored under the same conditions as the commercial pack blanched material (Table 1). The intermediate layer was highest in activity, followed by the kernel layer, with the COB fraction being lowest in activity. This pattern of lipoxidase distribution was the same in both corn varieties used in this study.

Positive peroxide numbers were obtained in the lipids from both varieties of corn. The extent of peroxidation and the acid numbers of the lipid matter extracted from the various tissues are shown in Table 1. The peroxide numbers were highest in the cob lipid and lowest in the kernel lipid. The effect of holding the material for five hours at room temperature was generally to reduce the peroxide numbers of the extracted lipids from the kernels and intermediate layers of both varieties and in the cob lipids of the Golden Cross corn. However, the peroxide number of the cob lipids for the Victory Golden samples showed an increase as a result of the holding treatment.

There were no striking varietal differences in the acid numbers of the lipids extracted from the various lyophilized tissues, either from the frozen samples or after holding them for 5 hours at room temperature.

The observation that under identical processing conditions one variety of corn contained larger amounts of residual lipoxidase activity than the other prompted a further investigation of the effects of certain processing conditions on retention of lipoxidase activity and quality during frozen storage. During the following season a series of over 250 samples were taken so that the effects of variety, maturity, blanching time and cooling time might be studied. In these studies the corn ears were sectioned as before and the intermediate layers were analyzed for residual lipoxidase content following frozen storage.

The effect of length of blanching time on residual lipoxidase activity for the 2 major varieties and of 4 experimental corn varieties is shown in Table 2. Although the 2 varieties were approximately equal in lipoxidase activity in the raw state, the lipoxidase in the Golden Cross was inactivated more readily than in the Victory Golden. With a blanching time of 4 minutes, the Golden Cross samples contained considerably less residual lipoxidase activity than the Victory Golden samples. No samples of Golden Cross corn, blanched for 6 minutes or longer, showed any residual lipoxidase activity, whereas with Victory Golden corn with 8 minutes blanching 3 out of 5 samples showed positive lipoxidase activity, and with a blanching time of 10 minutes 2 out of 9 samples still showed traces of residual lipoxidase. The pattern of residual lipoxidase activity of the four unnamed corn varieties closely resembled that of the Victory Golden corn.

Samples of Victory Golden corn, taken at intervals during the processing season, were subjected to different lengths of blanching and cooling times and analyzed for residual lipoxidase activity in the intermediate layer and for flavor score after being held in frozen storage (Table 3). As before, the shorter the blanching time, the greater the amount

TABLE 1

The relationship between lipoxidase activity and quality of commercial pack-corn-on-the-cob

Variety	Date Packed	Sample Number	Taste Score	Frozen									Held five hours at 21° C.								
				K Series			C Series			COB Series			K Series			C Series			COB Series		
				Lipoxidase [1]	Peroxide Number	Acid Number	Lipoxidase [1]	Peroxide Number	Acid Number	Lipoxidase [1]	Peroxide Number	Acid Number	Lipoxidase [1]	Peroxide Number	Acid Number	Lipoxidase [1]	Peroxide Number	Acid Number	Lipoxidase [1]	Peroxide Number	Acid Number
Golden Cross	Young	Raw	—	54	—	—	300	—	—	35	—	—	—	—	—	—	—	—	—	—	—
	Old	Raw	—	87	—	—	289	—	—	22	—	—	—	—	—	—	—	—	—	—	—
	8/16	1	Good	1	—	—							0	—	—						
	8/17	2	Good	0	1.2	7.3	6	3.2	5.7	No sample			0	1.1	7.1	7	2.4	7.1	No sample		
	8/18	3	Good	4	—	—							0	—	—						
	8/19	4	Good	13	0.7	7.0	6	1.9	6.4	3	32.8	28.4	2	1.3	7.2	0	0.7	5.2	6	33.7	31.8
	8/20	5	Good	3	—	—							0	—	—						
	8/21	6	Good	3	0.2	7.5	14	3.7	5.8	18	43.0	27.0	8	0	7.0	2	0	4.7	28	35.2	28.0
Victory Golden	Young	Raw	—	32	—	—	175	—	—	10	—	—	—	—	—	—	—	—	—	—	—
	Old	Raw	—	54	—	—	288	—	—	16	—	—	—	—	—	—	—	—	—	—	—
	8/29	7	Poor	0	0.6	7.0							0	1.9	8.0						
	8/28, 8/31	8	Poor	0	—	—	122	5.0	7.9	6	64.3	40.5	0	—	—	45	4.2	9.6	16	80.8	39.7
	8/30, 9/2	9	Poor	4	0.7	7.0							0	2.1	6.9						
	9/2, 9/3	10	Poor	0	—	—	32	5.3	9.3	20	62.2	34.2	0	—	—	9	—	8.8	15	81.4	36.3
	9/4, 9/5	11	Poor	0	—	—							0	—	—						
	8/29, 9/5	12	Poor	8	0	8.6	51	2.1	6.4	57	52.2	33.7	0	0.5	6.9	16	1.6	6.1	60	58.7	32.8
	8/30	13	Fair	0	—	—							0	—	—						
	8/29, 8/30	14	Fair	3	0.7	9.9	86	2.5	6.4	24	40.1	32.4	4	1.0	9.1	1	2.7	5.9	47	45.2	31.6
	8/30	15	Fair	0	—	—							2	—	—						
	8/31, 9/3	16	Fair	0	0.4	7.7	33	2.4	6.9	86	32.2	28.4	2	0.8	6.7	8	3.4	6.4	30	46.4	38.2

[1] μl. O_2 uptake per hour.

TABLE 2

Effect of blanching time on lipoxidase activity in frozen corn-on-the-cob

Variety	Blanch time min.	Number of samples tested	Lipoxidase activity [1]
Victory Golden	4	12	125 (33-202)
	6	5	65 (8-164)
	7	8	39 (0-101)
	8	5	6 (0-25)
	9.5	2	4 (0-7)
	10	9	1 (0-3)
	12.5	2	0 (0)
Golden Cross	4	9	75 (36-136)
	6	2	0 (0)
	7	5	0 (0)
	10	2	0 (0)
Four Unnamed Varieties	4	4	121 (93-155)
	7	4	10 (0-31)
	10	4	2 (0-7)

[1] µl. O_2 uptake per hour. Averages and extreme values, the latter in parentheses.

TABLE 3

Effect of processing conditions on lipoxidase activity and quality of frozen victory golden corn-on-the-cob

Sample number	Moisture %	Processing plant number	Blanching time min.	Cooling time min.	Lipoxidase activity C layer [1]	Flavor score
28	76	I	8	15	0	Good
29	76	I	10	15	3	Good
31	76	I	14	15	0	Good
82	71.5	II	4	15	185	Poor
83	71.5	II	6	15	164	Poor
89	71.5	II	7	6	101	Fair
216	68	I	4	4	202	Poor
213	68	I	4	15	73	Good
100	Old Corn	II	7	1	48	Good
101	Old Corn	II	7	4	63	Good
136	Old Corn	III	9.5	3	0	Good
137	Old Corn	III	9.5	9.5	7	Good
138	Old Corn	III	12.5	9	0	Good

[1] µl. O_2 uptake per hour.

of residual lipoxidase activity. For blanching times of equal length the samples cooled for longer times showed progressively less residual enzyme activity, generally proportional to the length of cooling time. Low flavor scores were characteristic of short blanching times, and were accompanied by relatively large amounts of residual lipoxidase. This was especially true early in the processing season of the less mature corn, i.e., that of higher moisture content. As the season progressed good flavor scores were obtained with somewhat shorter blanching times even though these ears contained moderate amounts of residual lipoxidase.

DISCUSSION

The commercial pack samples of Victory Golden corn used in this study were apparently underblanched with regard to maintenance of good quality in frozen storage, even though they had been blanched in flowing steam for ten minutes. This might have been inferred from the positive results of the qualitative tests for catalase and peroxidase, and was confirmed by the results of the taste tests. Corresponding samples of Golden Cross, although showing positive tests for catalase and peroxidase, were all scored *good* in the taste tests.

Positive tests for catalase and peroxidase in frozen corn do not necessarily indicate that the corn was inadequately blanched prior to freezing. Campbell has reported that strong positive catalase tests were obtained in cut corn scalded in flowing steam for as long as 20 minutes *(3)*. Mergentime *(14)* described the types of positive peroxidase reactions which could be observed in the skins and bases of the kernels of blanched corn, as well as false reactions due to the presence of cob tissue; these reactions were not necessarily indicative of underblanching. Phaff and Joslyn *(15)* have cautioned that the peroxidase test for blanching adequacy requires careful application, and Diehl *(4)* has stated that the qualitative test for catalase activity in blanched cut corn is not sufficiently reliable to warrant its use as an index of blanching.

Results obtained in the present study indicate that the distribution of lipoxidase in corn follows the same pattern as that observed in the various parts of barley grain by Franke and Frehse *(7)*. They showed that in barley the lipoxidase activity is contained primarily in the embryo. Fritz and Beevers *(8)* obtained active lipoxidase preparations from 3-day old hybrid corn seedlings from which the endosperm had been removed.

Varietal differences in residual lipoxidase activity can not be attributed to a difference in relative abundance of the enzyme in the raw corn, but more likely to a greater resistance to thermal destruction of the enzyme in the Victory Golden corn. Cob samples from raw corn differed from those of the blanched material in that the commercial pack samples consisted of cross-section portions of the cob, while the raw samples were tubular sections of the internal pith. The higher values for residual lipoxidase activity in the COB sections of blanched Victory Golden corn must have been due to higher initial activity in those regions of the cob nearest to the bases of the kernels. Variation from ear to ear within a processing batch and the certain amount of non-homogeneity occasioned during the sectioning of the samples gave rise to certain discrepancies in the analytical results, but the overall trends of the data were unmistakable.

It has been recommended that frozen corn-on-the-cob be partially thawed at room temperature prior to cooking, but that the thawing not be complete, lest the cooked product be soggy in texture and cobby in flavor *(6)*. To accentuate any deleterious effects which might result from this practice, portions of the samples used in this study were allowed to thaw completely and were held for a total time of five hours at room temperature.

The general lowering of lipoxidase activity observed as a result of holding the various corn tissues at room temperature was most likely due to denaturation of the enzyme. Lipoxidase is not generally regarded as

being very heat-stable *(1)*. The lipoxidase of snap beans was found to be very thermolabile. A blanch of 15 to 20 seconds in water at 88° C., was sufficient for complete inactivation of the lipoxidase in Tendergreen snap-beans, graded size 4, cut into one inch lengths *(19)*.

The peroxides found in the lipids extracted from the corn tissues may be due in part to autoxidation of the lipid matter. Positive peroxide values have been found in the lipid extracted from adequately blanched frozen cut corn of the Golden Cross variety. However, these peroxides were found only after prolonged storage of the cut corn at —17.8° C.; generally greater than one year's storage time being required before their detection was possible *(10, 12)*. A more logical explanation is that the peroxides resulted from the action of lipoxidase not completely inactivated during blanching.

The effect of holding the various tissues at room temperature for five hours was, in general, to cause a decrease in peroxide number of the lipids subsequently extracted therefrom. The increase in peroxide numbers of the lipids from the Victory Golden cobs may be taken as evidence of continuing lipoxidase action in these tissues during the holding treatment.

Siddiqi and Tappel *(16)* have shown that monocarbonyls are formed as a result of pea lipoxidase action at 0° C. They further postulated that the hydroperoxides formed under these conditions decomposed to form aldehydic products and polymers. These oxidation products were considered to be of greater importance in contributing to off-flavor than were the products resulting from autoxidation of fats during storage of frozen raw peas.

Differences in the physico-chemical structure of the two varieties might have a role in the continuation of lipoxidase action during freezing and storage. Tappel *et al. (18)* have reported that the lipoxidase system is particularly sensitive to change of state, and that at temperatures below 0° C., when ice formation occurs, the reaction rate in the solid state is less than one per cent of that in the supercooled liquid state, ascribed to lowered rates of diffusion of the reactants.

There were no striking varietal differences in the acid numbers of the lipids extracted from the various lyophilized tissues, either before or after holding for five hours at room temperature. The acid numbers from the kernels and intermediate layers were slightly higher than those normally found in blanched cut corn but were considerably smaller than those of raw cut corn of the Golden Cross variety after prolonged frozen storage *(10, 12)*. This would preclude the presence of any appreciable residual lipase activity. The relatively high acid numbers of the ether extracts from the cobs of both varieties may have been due to organic acids other than fatty acids.

The presence of substantial amounts of residual lipoxidase activity in the samples of Victory Golden corn, and only traces in the Golden Cross corn, was in general agreement with the organoleptic evaluation of the corn. It would appear that the off-flavors in the Victory Golden corn samples were due at least in part to the action of lipoxidase. The agreement between residual lipoxidase activity and low flavor score was particularly true for less mature corn. The observation that more mature corn scored *good* in the flavor tests despite moderate amounts of residual lipoxidase remains unexplained at the present time.

Results of this study lend additional support to the theory that the major locus of off-flavor development in certain frozen underblanched vegetables is in the lipid fraction, and that off-flavor production is associated with deleterious changes brought about by enzymes acting upon the components of the lipid fraction *(11, 20, 21, 22)*.

SUMMARY

Commercial pack Victory Golden and Golden Cross corn-on-the-cob was assayed for residual lipoxidase activity and flavor score following six months storage at —17.8° C. The Victory Golden corn showed residual lipoxidase activity and had developed off-flavor during frozen storage.

In the Victory Golden corn ears the area of greatest lipoxidase activity was the intermediate layer containing the germ; moderate activity was found in the cobs; and only trace amounts of lipoxidase were found in the kernels.

Positive peroxide numbers were observed in the ether-extracted lipids from all sections of both varieties of corn.

Holding the various tissues at 21° C. for 5 hours prior to analysis served generally to reduce both the lipoxidase activity and the peroxide number of the lipid matter.

There was no evidence of lipase action as measured by the determination of acid numbers of the ether-extracted lipids.

The distribution of lipoxidase activity in raw corn ears was measured and found to be substantially the same in both corn varieties.

The effects of maturity, blanching time, and cooling time on residual lipoxidase activity and flavor of Victory Golden corn were investigated.

The off-flavors which developed during frozen storage of inadequately blanched corn-on-the-cob were considered to be due in part to the action of residual lipoxidase.

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CATALASE AND PEROXIDASE ACTIVITY IN RAW AND BLANCHED SOUTHERN PEAS, *VIGNA SINENSIS*

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The term, Southern peas, includes several varieties of the species *Vigna sinensis*. The several varieties are commonly grouped under one of the following classifications: field peas, crowder peas, blackeye peas. Southern peas are grown rather extensively in some areas of Texas, Georgia, Virginia, and of other Southeastern states. Some of the crop is sold in the fresh market but a large portion is canned or frozen.

The work of Joslyn and Cruess *(5)* in 1929 and of other workers in later years, demonstrated the need for blanching vegetables to be preserved by freezing. Raw or underblanched vegetables discolor, lose flavor, and develop off odors during frozen storage. It is now widely recognized that the loss of quality that unblanched or underblanched vegetables experience during freezing storage is caused by enzymatic action. The enzyme system or systems directly responsible for the deteriorative changes have not been identified. However, it has been found that the enzymes, or enzyme systems, peroxidase and catalase, present in all vegetables, are among the most heat resistant enzymes. When catalase and peroxidase are found to have been inactivated by heating, the enzymatic processes in the vegetable are inhibited, as other enzymes generally would have also been inactivated because they are less heat resistant.

Wagenknecht and Lee *(10)* have studied the effect of added catalase, peroxidase, lipoxidase, and lipase to frozen peas. They found that lipase and lipoxidase produced more pronounced off-flavors than catalase and peroxidase, and that the addition of all four of these enzymes resulted in changes in green color.

Balls *(2)*, Schwimmer *(8)*, Guyer and Holmquist *(3)*, and Vetter, Nelson, and Steinberg *(9)* have studied the conditions under which regeneration of peroxidase occurs in several different vegetables. In general, it has been found that in order to irreversibly inactivate peroxidase, the time-temperature conditions used must be more intense that the minimum time-temperature conditions needed to inactivate the enzyme. Therefore, enzyme regeneration must be considered an important factor in blanching vegetables for freezing and in the sterilization of canned foods, especially when high temperature-short time processes are used.

The objectives of the work reported here were: (a) to determine the blanching time required to inactivate catalase and peroxidase in Southern peas; (b) to determine whether any enzyme activity regeneration would take place during frozen storage of Southern peas under the conditions of the experiment.

EXPERIMENTAL METHODS

Five varieties of Southern peas were grown for this experiment in 1955 at the Horticultural Farm, Virginia Agricultural Experiment Station, Blacksburg, Virginia.

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The varieties and number of samples of each variety used were: Dixie Lee (3 samples), Brown Crowder (1 sample), Cream (2 samples), Blackeye (3 samples), and Purple Hull (4 samples). Samples of each variety were picked on different dates. All the varieties mentioned above are important processing varieties.

The Southern peas were picked at a stage of maturity when the pods were light green or had brown as well as green areas. The peas were succulent, nearly fully-grown, and the color ranged from a bright green to a very light green. This stage of maturity was selected on the basis of previous work *(6)*. Within 2 hours after picking, the peas were shelled in a Dixie Junior Pea Sheller and placed in hermetically sealed glass jars. Within one hour after shelling, the peas were placed in storage at 34° F. (1.1° C.). In the course of the same day, the peas were taken out of refrigerated storage and the experimental samples prepared as follows. Peas from each picking of each variety were divided in 5 portions. One of these portions was used to prepare the unblanched and blanched samples for the 0 months storage time enzyme activity determinations, which were performed the same day. Another portion was subdivided in approximately 75-g. portions and packaged in ½ pint glass jars, hermetically sealed, and stored at 0° F. (—17.8° C.). The other 3 portions of each sample were blanched by immersing them in constantly boiling water (212° F, 100° C.) for, respectively, 1, 2, and 3 minutes. For blanching, the peas were placed in cheese cloth bags. Approximately one pound of peas was blanched at a time. The circulation of water among the peas was promoted in order to give the individual peas an even blanch. After the blanching period the samples were cooled by immersing them in water at 65° F. (18.3° C.) for 5 minutes. After cooling, the samples were drained for 5 minutes, dried between sheets of filter paper, subdivided in approximately 75-g. portions, placed in ½ pint glass jars, hermetically sealed, and placed in frozen storage at 0° F. (—17.8° C.). They were kept stored at 0° F. until the enzyme activity determinations were made.

The enzyme extract was prepared as follows. For each enzyme activity determination, one 75-g. sample was used. It was allowed to thaw until the peas would seperate from each other. Then 50 g. of peas were weighed, and the sample preparation continued using the procedure given in *Methods of Analysis* of the A.O.A.C. *(1)* for the catalase determinations, and the method of Reddi *et al. (7)* for the peroxidase samples.

The method followed for determining catalase activity was that given in *Methods of Analysis* of the A.O.A.C. *(1)*. For peroxidase activity the ascorbic acid oxidation procedure of Joslyn *(4)* was employed.

Determinations of the activity of catalase and of peroxidase were made on pea harvest day (0 months), and after 3, 7, and 29 months of storage at 0° F. (—17.8° C.). The enzyme activity determinations were run on the unblanched samples, and on the samples that had been blanched for 1, 2, and 3 minutes. All determinations were made in duplicate.

RESULTS

Results of the catalase and peroxidase activity determinations are given in Tables 1 and 2. Catalase activity is expressed as the K_t value, which is an expression of the catalase content of the sample and, therefore, of its catalase activity per unit of time. Pure catalase has a K_t value of 40,000 to 60,000, depending on source *(1)*. Peroxidase activity is expressed in terms of mols of ascorbic acid oxidized by peroxidase during the reaction time. The amount of ascorbic acid oxidized is directly proportional to the content of pure peroxidase of the sample, and to the peroxidase activity per unit of time.

Tables 1 and 2 show that after a one-minute blanch in boiling water approximately 70 to 90% of the peroxidase and 80 to 100% of the catalase activity had been inhibited. After a 2-minute blanch, approximately 90 to 100% of the peroxidase and 98 to 100% of the catalase activity had been inhibited. After a 3-minute blanch, 98 to 100% of the peroxidase activity had been inhibited. No significant catalase or peroxidase regeneration was observed after any of the 0° F. (—17.8° C.) storage periods.

DISCUSSION

The results reported here indicate that, in order to completely inhibit enzyme action, Southern peas should be blanched for 3 minutes at 212° F. (100° C.) or be given an equivalent blanching at another temperature. A blanching of 2 minutes at 212° F. completely inactivated catalase and in-

TABLE 1

Catalase activity of Southern peas before and after storage at 0° F. (−17.8° C.).

Variety	Number of samples	Before freezing			3 Months storage		
		Blanching time			Blanching time		
		0 Min.	1 Min.	2 Min.	0 Min.	1 Min.	2 Min.
		K_f	K_f	K_f	K_f	K_f	K_f
Dixie Lee	3	.082 [1]	.004	.000	.082	.001	.000
Brown Crowder	1	.140	.008	.000	.164	.001	.000
Cream	2	.139	.000	.000	.139	.003	.000
Blackeye	3	.074	.002	.000	.087	.005	.000
Purple Hull	4	.238	.003	.000	.224	.000	.000

Variety	Number of samples	7 Months storage			29 Months storage		
		Blanching time			Blanching time		
		0 Min.	1 Min.	2 Min.	0 Min.	1 Min.	2 Min.
		K_f	K_f	K_f	K_f	K_f	K_f
Dixie Lee	3	.076	.007	.000	.080	.010	.001
Brown Crowder	1	.169	.016	.000	.135	.009	.000
Cream	2	.145	.002	.000	.134	.007	.001
Blackeye	3	.080	.005	.001	.063	.008	.000
Purple Hull	4	.234	.008	.001	.207	.006	.001

[1] All values are average of number of samples indicated.

TABLE 2

Peroxidase activity of Southern peas before and after storage at 0° F. (−17.8° C.) expressed in mols ascorbic acid oxidized

Variety	Number of samples	Before freezing				After 3 Months' storage			
		Blanching time				Blanching time			
		0 Min.	1 Min.	2 Min.	3 Min.	0 Min.	1 Min.	2 Min.	3 Min.
Dixie Lee	3	.058 [1]	.015	.002	.000	.052	.011	.011	.000
Brown Crowder	1	.049	.008	.001	.000	.041	.008	.001	.000
Cream	2	.095	.018	.004	.000	.105	.013	.004	.000
Blackeye	3	.037	.008	.002	.000	.042	.006	.002	.000
Purple Hull	4	.058	.012	.002	.000	.057	.012	.002	.000

Variety	Number of samples	After 7 Months' storage				After 29 Months' storage			
		Blanching time				Blanching time			
		0 Min.	1 Min.	2 Min.	3 Min.	0 Min.	1 Min.	2 Min.	3 Min.
Dixie Lee	3	.005	.013	.002	.000	.058	.015	.002	.000
Brown Crowder	1	.047	.008	.002	.000	.042	.011	.001	.000
Cream	2	.095	.012	.002	.000	.091	.018	.003	.000
Blackeye	3	.036	.005	.001	.000	.043	.005	.000	.001
Purple Hull	4	.058	.011	.002	.000	.054	.007	.001	.001

[1] All values are average of number of samples indicated.

hibited 90 to 100% of the peroxidase activity. The latter blanching conditions may be considered adequate in some instances, as the respiratory activity and other enzymatic processes would be almost completely stopped by an exposure of the Southern peas to 212° F. for 2 minutes. These results are applicable only to the pea varieties included in this work. However, there is a high degree of probability that the catalase and peroxidase activity of other Southern pea varieties would be inhibited in approximately the same degree as that reported for the five varieties tested, when similar blanching conditions are applied.

SUMMARY

Quantitative catalase and peroxidase activity determinations were made in unblanched and in blanched samples of 5 varieties of Southern peas, *Vigna sinensis*, after 0° F. (−17.8° C.) storage periods of 0, 3, 7, and 29 months. A one-minute blanch in boiling water (212° F., 100° C.) inhibited approximately 70 to 90% of the peroxidase and 80 to 100% of the catalase activity. After a two-minute blanch, approximately 90 to 100% of the peroxidase and 98 to 100% of the catalase had been inhibited. A three-minute blanch resulted in 98 to 100% inactivation of the peroxidase activity. No significant catalase or peroxidase regeneration was observed after the 0° F. storage periods.

Acknowledgments

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OBSERVATIONS ON THE HISTOLOGY AND TEXTURE OF ELBERTA PEACHES FROM TREES OF HIGH AND LOW LEVELS OF NITROGEN NUTRITION

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Commercial canning of freestone peaches, such as Elbertas, often results in tissue sloughing and a ragged appearance by comparison with the firmly coherent texture of canned clingstone varieties. In some areas, however, plantings of freestone varieties predominate and have increased to the point that quality improvement in their use in the canned fruit market has received serious consideration. This is the situation in the state of Washington *(10)* where it has been found that the canning quality of Elberta peaches can be improved by orchard application of high levels of nitrogen-rich fertilizers *(5, 13)*.

Quality requirements for canned freestone peaches differ from those for market of the fresh fruit. The influence of nitrogen on tree growth and on yield and quality of the fresh fruit have been generally recognized at least since 1930-1931 when Addoms, *et al. (1, 4, 11)* described in detail the composition and fresh fruit qualities of high and low nitrogen, New Jersey — grown Elberta and Shipper Cling peaches. These authors considered that the high nitrogen Elberta fruit did not develop a desirable flesh color until too soft for shipment and table use. Peaches representing low to medium nitrogen levels, however, tended to be slightly elongate in shape, matured and colored more rapidly, and could be harvested earlier at a firmer texture for shipment. In general, it has been recognized that trees of high nitrogen nutrition show lush, vigorous growth and bear larger, more rounded fruit than do low nitrogen trees which have comparatively sparse growth and smaller leaves.

Very similar differences in fresh fruit qualities were found by Carter *et al.* between Elberta peaches from trees of high and low levels of applied nitrogen grown in soil management plots in Washington State *(5, 13)*. However, fruit from trees receiving applied nitrogen fertilizers in amounts necessary for maximum yields was considered to be superior in canning qualities to that representing low to deficient nitrogen conditions. When canned, the high nitrogen fruit showed a firm, finely coherent texture, good color, and was less astringent that the coarse, often stringy and ragged fruit from trees of low nitrogen status.

The fine and coherent versus the coarse and ragged texture of these canned Elberta peaches suggested that cellular distinctions may result from different levels of applied nitrogen. The possibility was investigated and comparative histological studies were made on selected samples of these Washington State peaches in both the canned and the fresh conditions.

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SAMPLES AND NUTRITIONAL HISTORY

All fruit used in these studies was obtained from trees grown in the above mentioned soil management plots at the Irrigation Experiment Station, Prosser, Washington[b]. In brief, these plots are located on a fine sandy loam of low nitrogen. Some of the plots with and without added nitrogen were under clean cultivation management with an August-planted rye grass crop which was disked under in late May. The fruit compared in the present studies represents upper and lower levels of nitrogen nutrition previously established and described for these plots. Leaf nitrogen was used as an index of individual tree response to nitrogen fertilization and had been found also to correlate with various canning qualities *(5, 13)*.

Whole fresh-frozen and canned halves of Elberta peaches of the 1956 season and fresh peaches of the 1957 and 1958 seasons were shipped to this Laboratory from the Prosser Station. The low nitrogen fruit was harvested from trees receiving no added nitrogen in 1956; the high nitrogen fruit of that season was from trees receiving 2.5 and 2 pounds actual nitrogen annually per tree. Fruit of the 1957 season was from these same trees. Leaf nitrogen for the low nitrogen status was 1.7% and averaged 3.1% for the high nitrogen trees.

It was believed that by 1958 the roots of some of the low nitrogen trees had sufficiently extended that they were receiving some nitrogen from joining areas of high nitrogen application. The low nitrogen fruit of the 1958 season was harvested from such a tree while that of high nitrogen status was from a tree receiving 4 pounds added actual nitrogen annually.

Season variation in fruit size of the peaches received at this Laboratory was apparent for each nitrogen status. Yields of the 1957 season were abnormally low because of adverse weather and both the high and the low nitrogen fruits were larger than those of the 1956 and 1958 seasons. Nevertheless, the 1957 fruit exhibited the respective shape and color characteristics of high and low nitrogen peaches of the 1956 season. That is, the low nitrogen fruit tended to be elongate or lenticular as compared with the slightly larger, well-rounded peaches of high nitrogen status. On the other hand, the 1958 season was characterized by a heavier than normal fruit set and larger yields than in 1956; shape differences in the fruit were less marked and the 1958 fruit was of smaller average size. The 1958 season was warmer than normal. Mean monthly temperatures were 71, 74, and 72° F. for June, July and August, respectively, as compared with 62, 72 and 69° for the 1956 season and 65, 71, 69° F. for 20-year averages.

The fruit of the 1956 and 1958 seasons was harvested on a date when it was considered to be at a maturity which would reach a uniform yellow ripeness after 5 to 6 days of post-harvest ripening. It had been previously established *(5, 13)* that an average pressure test of 5 to 6 pounds with a 5/16-inch plunger on pared cheeks was indicative of such a harvest maturity. In 1956 peaches of high nitrogen status required about 10 days longer on the tree to reach this harvest maturity than did the low nitrogen fruit. Harvest of the high nitrogen fruit in 1958 was only 6 days later than for the low nitrogen peaches.

Fresh samples of the 1957 season were harvested to provide an extended range of maturities without regard to the pressure test since yields were too low for consideration for canning investigations. Some immature peaches of each nitrogen status also were collected in 1958 several weeks prior to predicted harvest. In addition, leaf buds and very young fruits were collected and preserved in histologic fluids for developmental studies the results of which will be described elsewhere.

All fresh samples were carefully packed and shipped to this Laboratory where they were received in good condition 5 to 6 days after harvest. As received, most of the mature peaches appeared to be at about optimum ripeness for canning. All canned samples examined here were processed at Prosser, Washington, as previously described *(5, 13)*.

HISTOLOGICAL METHODS

Thick sections cut on a sliding microtome without prior histological preparation and embedding of the peaches were found to be suitable for most observations on the canned, fresh-frozen, and fresh fruit. These sections were cut thick enough (200 to

[b] The cooperation of Dr. A. M. Neubert of the United States Fruit and Vegetable Products Laboratory, and of Dr. Proebsting, of the Irrigation Experiment Station, Prosser, Washington is greatly appreciated.

320 mμ) to ensure the presence of intact cells throughout the section. Sections cut in planes radial to the fruit and in the region of greatest cheek circumference were used for measurements of cell size; both radial and tangential sections were used for observations on diameters and distributions of the strands of vascular bundles.

Microscopic measurements of cell size were made with a calibrated eye-piece micrometer at 100X magnification. An outer flesh region about ⅛ to ⅜ an inch beneath the skin was selected and only the spherically shaped flesh parenchyma cells in this area were measured. This selection was based upon consideration of parenchyma cell shape variations within the peach flesh. For example, some of the parenchyma cells bordering the vascular bundles of ripe peaches are irregularly elongated and most of the parenchyma cells of the inner flesh towards the pit are elongated radially to the fruit. On the other hand, most of the cells between the vascular bundles of the outer flesh tend to be isodiametric *(15, 17, 18, 19)* and may be treated as spherical shapes which provide a convenient index for size difference. In addition, the spherical cells of the outer flesh may be further separated into two classes: (1) larger ones closely associated with the vascular bundles and (2) patches of smaller cells between the vascular bundle areas.

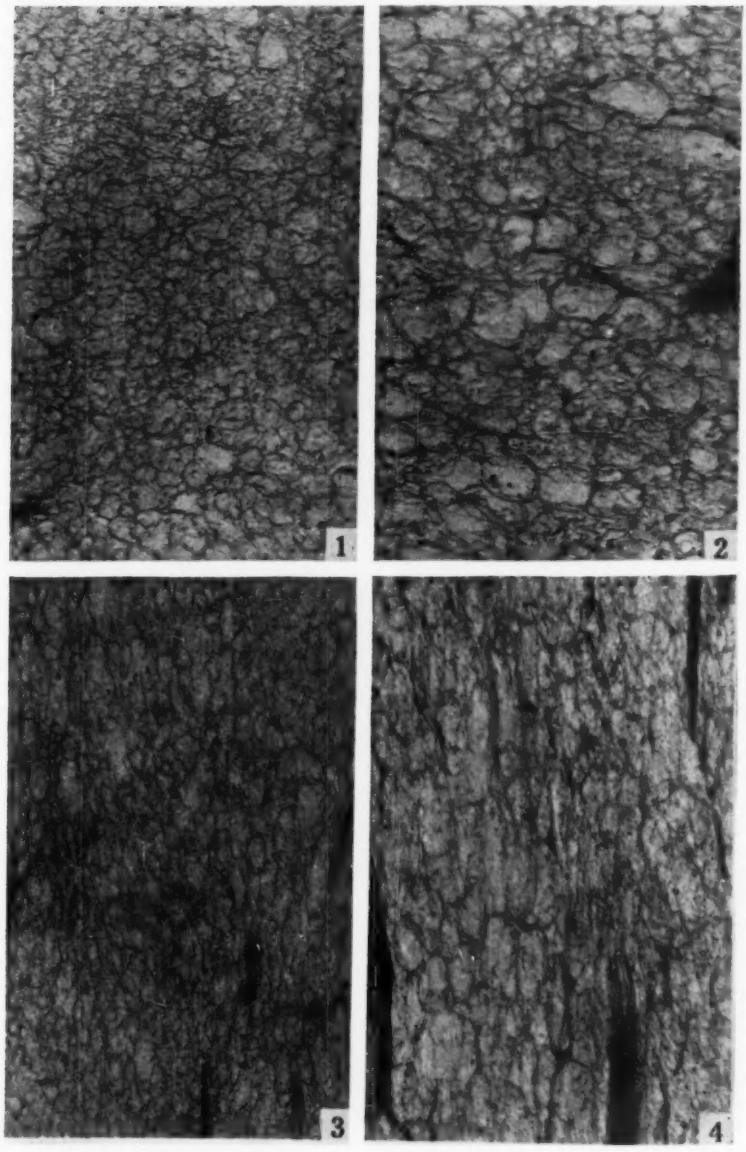
Cell measurements for peaches of the 1956 season were made without regard to differences in these areas in the outer flesh. Measurements made for peaches of the 1957 and 1958 seasons, however, were according to the following classes: Class 1 — all spherical cells of the outer flesh (as in the 1956 season); Class 2 — smaller spherical cells between the vascular bundles of the outer flesh; Class 3 — larger spherical cells of the vascular bundle areas; Class 4 — elongated parenchyma cells of the inner flesh according to widths and lengths. All measurements were made in separate series for each class and, as nearly as possible, all cells were measured in sequence as they appeared under the scale of the eye-piece micrometer in order to avoid sampling bias. Measurements also were recorded separately for individual fruits of different sizes within each nitrogen status. One hundred or more spherical cells per section were measured for each of the cell classes 1, 2 and 3 in at least 2 sections each of 2 to 5 fruits of similar size in each nitrogen status. Thus, each sampling consisted of 1000 or more cells. Only 300 to 500 elongated inner flesh cells per fruit were measured for Class 4. Also, only intact cells were measured at the plane of media optical focus in all samplings.

Because of the marked variation in size of the spherically shaped parenchyma cells of the outer flesh several checks were made to validate this sampling method. It was found that for Class 1 which showed the greatest size variation, successive groups of 100 measured cells out of a sampling of 1000 cells gave averages which ranged between 5 and 10% difference from the average obtained from the total measured samples. For Class 2 cells which showed the greatest uniformity in size, successive groups of 50 measured cells gave averages which usually were within 5 percent of the average obtained from the total Class 2 sample. This was better than had been anticipated and it was possible to utilize smaller samplings of 100 to 200 cells for verifications on replicate, individual fruits.

Fresh sections of peaches of the 1958 seasons also were tested histochemically for catechol tannins. The test used is based on the nitroso reaction of phenolics with nitrous acid and the intensities of positive color development in the sections were measured photometrically and used as a qualitative index of the relative amounts of catechol tannins present *(16, 18)*. In this test equal volumes of aqueous 10% solutions of acetic acid and sodium nitrite and 20% urea are mixed in a petri dish and the sections added. After 3 minutes reaction the sections are transferred to 2 *N* sodium hydroxide in aqueous solution. A characteristic, intense cherry-red color develops in the presence of catechol, chlorogenic acid and similar catechol derivatives and the test is very sensitive. The intensities of this color, recorded as reflection densities of the test sections against that of a control section, can be measured photometrically in the same manner as spots developed in paper chromatography *(18)*. In histological application, sections of the same uniform thickness are essential and an average is obtained from 5 or 10 readings per section in 5 sections per fruit sample.

RESULTS

Figures 1 to 4 illustrate the differences observed in cell size between the canned high and low nitrogen fruit of the 1956 season. The smaller parenchyma cells of the outer (Fig. 1) and inner (Fig. 2) flesh regions distinguish the high nitrogen, finely texture fruit from the larger celled, low nitrogen and coarsely textured product (Figs.



Figures 1-4. Photomicrographs of sections of canned high- and low-nitrogen Elberta peaches (1956 season) showing overall differences in cell size of flesh parenchyma. x 40.

Figure 1. High nitrogen, outer flesh; Figure 2. Low nitrogen, outer flesh.

Figure 3. High nitrogen, inner flesh; Figure 4. Low nitrogen, inner flesh.

3 and 4, respectively). These differences were observed between all canned samples of high and low nitrogen status examined — about 80 to 100 fruit halves for each group. In canned appearance the low nitrogen fruit consistently appeared to be inferior; the cut edges of the halves varied from slightly to definitely ragged and the strands of vascular bundles appeared to be more pronounced than in the high nitrogen fruit. No evident tissue sloughing was observed in the high nitrogen samples; the edges were clean-cut and the flesh was solid — characteristics usually ascribed to canned clingstone peaches.

Although the vascular bundle strands of the high nitrogen fruit appeared to be less pronounced than those of the low nitrogen status, no consistent differences in width measurements could be found. If such a difference existed it probably could be detected only through a more complete structural analysis of the vascular systems of these fruits and comparison of exactly comparable bundles. These vascular systems consist of complexly branching and anastomosing strands and degree of complexity could, conceivably, influence bundle width. Provisionally, however, it appears that difference in apparent "stringiness" between high and low nitrogen canned peaches here may be an over-all visual effect — that is, the larger flesh cells of the low nitrogen peaches render the narrow-celled vascular bundles more noticeable than they appear to be in the smaller-celled high nitrogen fruit.

These observations agree with the judged firmness and textural quality scores previously reported for canned high and low nitrogen Elberta peaches from the same soil management plots for the same and previous seasons *(5, 13)*, some of which are represented in the present samples.

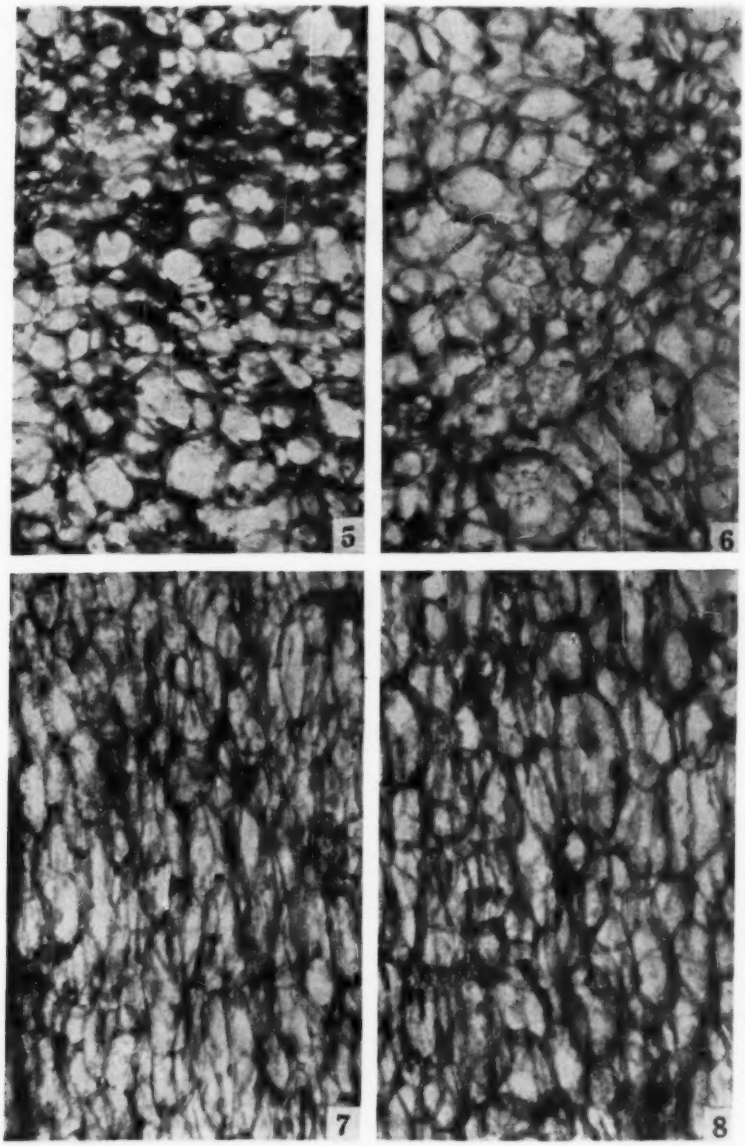
Similar differences in parenchyma cell size were found in fresh sections of the 1957 fruit and in sections from frozen 1956 samples replicate to the canned product (Figs. 5 to 8). Additionally, the inner flesh region of radially elongated cells in the low nitrogen fruit usually was proportionately more extensive and the outer region of spherical flesh cells less extensive than in the high nitrogen fruit. Thus, it would appear that the high nitrogen fruits not only contained the smaller cells but also contained more extensive development of the smaller-celled tissue regions than the low nitrogen fruit for these two seasons. This would appear to relate to the previously mentioned differences in fruit shape between high and low nitrogen peaches.

The magnitude of cellular difference between these peaches is more exactly illustrated by the measurements made on their spherically shaped parenchyma cells in their outer flesh. Representative distributions of cell size are shown in Figure 9 for 3 fruit sizes each of ripe high and low nitrogen peaches of the 1956 season. Although essentially the same range in cell size was found in both high and low nitrogen fruits, definitely more smaller cells were found in all high nitrogen than in low nitrogen fruit. This again appears to relate to the differences in cell size are pronounced is further shown in Table 1 which compares spherical cells of the low nitrogen fruits as to average cell diameters and volumes of their average size cells. The spherical cells of the low nitrogen fruit for the 1956 season — and also for the 1957 season, as shown in Table 2. In other words, for a given tissue volume, the outer flesh of the high nitrogen fruit contains nearly twice the number of spherical parenchyma cells as does the low nitrogen fruit. In microscopic counts of cell numbers over a given linear distance of section it was

TABLE 1

Average size of spherical parenchyma cells (Class 1) in outer flesh of individual Elberta peaches of high and low nitrogen status for the 1956 season

Nitrogen status	Cheek circumference (inches)	Cell Size	
		Average diameters (microns)	Volume for average size (microns ^{3})
High Nitrogen	7	131	11.8×10^5
	8	125	10.2×10^5
	9	135	12.9×10^5
Low Nitrogen	7	156	19.9×10^5
	8	161	21.8×10^5
	9	160	21.3×10^5



Figures 5 to 8. Photomicrographs of fresh sections of high- and low-nitrogen Elberta peaches (1957 season) comparing cell sizes of flesh parenchyma cells. x 80.

Figures 5 and 6. Outer flesh of high and low nitrogen fruit, respectively.

Figures 7 and 8. Inner flesh of high and low nitrogen fruit, respectively.

TABLE 2

Average parenchyma cell size and histochemical test results for individual Elberta peaches of high and low nitrogen status of the 1957 and 1958 seasons

Nitrogen status and season	Harvest date and maturity when tested *	Cheek circumference (inches)	Nitroso color intensity	Cell size					Volume of average size spherical cells (microns) Class 1
				Average diameters of spherical cells (microns)			Average dimensions of inner flesh cells (microns)		
				Class 1	Class 2	Class 3	Widths	Lengths	
High nitrogen									
1957	Sept. 9								
	ripening	8.5	—	133	109	191	—	—	12.3×10^5
1958	Aug. 11								
	immature	6.0	0.95	—	86	128	74	198	—
	immature	6.0	0.99	—	87	135	—	—	—
	Sept. 3								
	ripening	8.75	0.33	120	107	172	89	416	9.05×10^5
	ripe	7.5	0.29	123	108	172	86	392	9.6×10^5
	ripe	7.75	0.24	113	97	156	—	—	7.6×10^5
Low nitrogen									
1957	Sept. 9								
	ripening	8.5	—	158	120	208	—	—	20.6×10^5
1958	Aug. 11								
	immature	6.75	0.74	—	93	131	72	199	—
	immature	7.25	0.65	—	104	147	101	326	—
	Aug. 26								
	ripe	7.5	0.51	136	118	160	106	399	13.2×10^5
	ripe	8.25	0.51	128	110	161	—	—	12.4×10^5
	ripe	8.25	0.49	127	—	—	—	—	11.3×10^5

* All 1958 samples received 6 days after harvest; most of the mature fruit had ripened to canning maturity. Nitroso test was conducted and sections prepared on 7th day after harvest. Cell measurements were made on sections temporarily stored at 5 percent formalin solution.

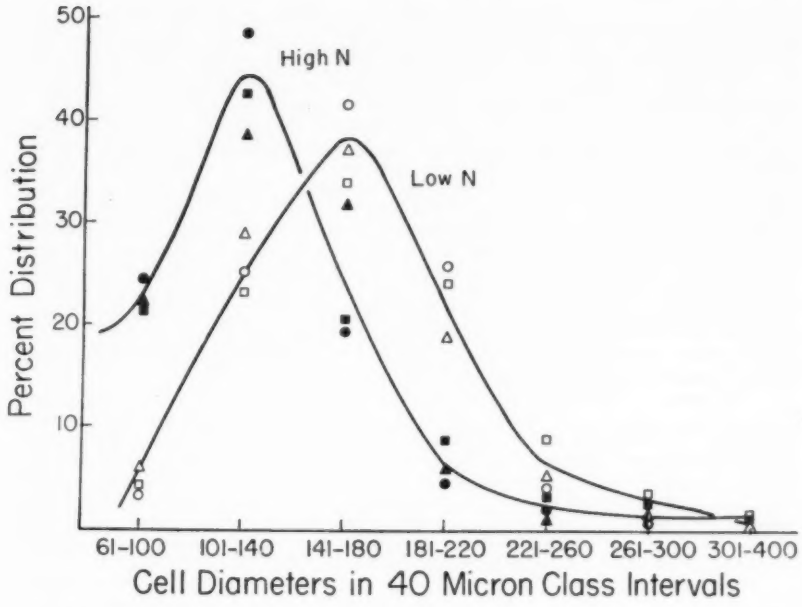


Figure 9. Distributions of cell size in high and low nitrogen peaches (1956 season).

△ ▲ — **Fruit 7 inches circumference.**
○ ● — **Fruit 8 inches circumference.**
□ ■ — **Fruit 9 inches circumference.**

found that the cubes of numbers obtained for high and low nitrogen peaches were approximately in a 5:3 ratio.

The fruit of the 1958 season showed less difference in cell size than was obtained in the outer flesh of the high and low nitrogen peaches of the 2 prior seasons (Table 2). In addition, all fruits averaged smaller size cells in 1958 than in 1956 and 1957. This seasonal difference seems noteworthy in view of the fact that the high nitrogen fruit examined here for the 1958 season was from a tree which had received twice as much applied nitrogen as did the high nitrogen trees from which the 1956 and 1957 peaches were harvested. However, as previously discussed, the mean monthly temperatures and fruit yields in the 1958 season were higher and both the high and low nitrogen peaches were of smaller average size than in 1956. Likewise, as already mentioned, it was suspected that the root systems of the tree of low nitrogen status for 1958 had sufficiently extended that it was receiving some nitrogen from adjacent areas of high nitrogen application. Nevertheless, average cell size in the spherical cell classes 1 and 2 of the mature fruit of the 1958 season was larger in the low than in the high nitrogen fruit and replicate check measurements failed to reveal evidence of overlapping in these averages. No significant differences were found in size of either the Class 3 spherical cells or the elongated cells (Class 4) of the inner flesh for the 1958 fruit; although, in 1956 and 1957, these cells were definitely of smaller dimensions in the high than in the low nitrogen peaches — as illustrated in Figures 3, 4, 7 and 8.

No significant differences in cell size were found between mature peaches of different size within each nitrogen status for a given season. As shown in Table 1 for the 1956 season and in Table 2 for the 1958 season, different size fruits within each nitrogen status had about the same average spherical cell size; moreover, those with slightly smaller

average size were sometimes the larger fruit. Cell numbers have been found to have greater influence than cell size upon mature fruit size of apples of a given variety *(3)*. It would appear that this is also true of peaches within a given culture status and season.

The immature peaches of the 1958 season listed in Table 2 also show differences which relate to their nitrogen status. It is well recognized that high levels of applied nitrogen fertilizers to peach trees results in delayed fruit maturation. The August 11 collection shows the high nitrogen fruit to be of smaller size and to contain smaller growing parenchyma cells than the low nitrogen fruit. This reflects difference in maturation rates. Further indication of slower maturation in the high than in the low nitrogen fruit is shown by the more intense color tests for catechol tannins in the high than the low nitrogen peaches on August 11; the greater intensity indicating the higher tannin content. In other studies on tannins and fruit development, California peaches were investigated for several seasons *(18)*. It was found that, as cell divisions ceased and cell enlargement began in early phases of fruit growth, large increases in catechol tannins could be revealed histochemically. Such increases in phenolics, perhaps principally in chlorogenic acid, are associated with mechanisms of cell growth in plant tissues. They apparently relate to the inhibition of indol-acetic acid oxidase by phenolics which frees the auxin necessary for cell enlargement to occur *(14, 18)*.

The mature Washington State Elberta peaches listed in Table 2 for the 1958 season likewise show differences in tannin test color intensity between high and low nitrogen conditions. Intensities of color test were consistently lower in the ripe high nitrogen than in ripe low nitrogen peaches. Carter *et al. (5, 13)* in their earlier studies on the canning qualities of fruit from the same orchard found that the high nitrogen fruit usually was less astringent than the low nitrogen fruit. The above studies on California peaches *(18)* revealed histochemical test differences between varieties which apparently related to their degree of astringency. In general, however, all ripening fruit showed decreases in test color intensities from those obtained in the immature fruit.

DISCUSSION

The relation of nitrogen status to texture and other canning qualities of Washington State Elberta peaches has been previously established and discussed in detail *(5, 13)*. These present observations on peaches of the same and similar cultural histories from the same orchard serve to explain the physical basis for the fine and coarse textural appearances, respectively, of the high and low nitrogen fruit. That the smaller average cell size of the high nitrogen fruit is a result of excess nitrogen application seems well established. The high nitrogen fruit consistently showed smaller cell size characteristics than the low nitrogen fruit over 3 successive seasons. Moreover, this nitrogen effect was of sufficient order that extreme seasonal variations, as in 1957 and 1958, did not obscure this basic difference.

To the author's knowledge, these are the first recorded observations of cellular differences between fruits from trees of different nitrogen nutrition. Some consideration of the literature on the influence of nitrogen on growth of plant tissues is therefore pertinent. Responses of vegetative tissue growth to different levels of nitrogen have been widely investigated. In brief, the apical meristems or embryonic growing points of leafy shoots utilize large quantities of carbohydrates and nitrogenous compounds in the production of new cells and organs. The availability of these materials influences both size and number of new cells and organs — and also other physical characteristics of the tissues *(8)*.

The early work of Kraus and Kraybill *(7)* with tomato plants established 4 metabolic conditions: (a) Plants with a very low ratio of available carbohydrates to available nitrogen were vegetatively weak, succulent, and unfruitful. (b) Those with a nominally low ration of available carbo-

hydrates to available nitrogen showed vigorous vegetative growth but were unfruitful. (c) Those with an intermediate ratio of available carbohydrates to available nitrogen showed good growth and fruitfulness. (d) Plants with a high ratio of available carbohydrates to available nitrogen had slender, woody growth and small, woody or tough fruit. Analyses showed the nitrogenous content to vary inversely with the reverse (insoluble) carbohydrates.

In more recent investigations, increased nitrogen application was found to result in increases in the number of leaves produced by the apical meristems of several plants — and also increased numbers of cells per leaf *(9)*. Increased leaf surface, due to increased cell production, and increases in both stem length and leaf size, due to larger cells at full organ maturity, also have been found to result with increased nitrogen availability with kale *(2)*. Relationships between fruit growth, cell size and nitrogen metabolism also have been investigated with apples but without regard to experimentally varied nitrogen availability *(12)*.

The formative stages of organ growth and tissue differentiation are particularly pertinent to an understanding of the influence of nutrition. In perennial species, for example, the flower or fruit buds are histogenetically differentiated by transition from the vegetative apical meristems of leafy shoot buds during the year preceding the fruit growth season *(6, 8, 18)*. Consequently, conditions which influence numbers of cells produced by these meristematic tissues in forming new vegetative organs also influence cell numbers in the fruit.

The formative or histogenetic phases of fruit growth have been widely studied for peaches by Addoms *et al. (1)* and others *(15, 18, 19)* — and also for other fruits *(17, 20)*. Although Addoms and co-workers included a consideration of nitrogen status, they did not report cell size distinctions between high and low nitrogen peaches and their histological descriptions mainly compare the structure of New Jersey-grown Elberta and Shipper Cling varieties as to possible cellular causes for the different texture qualities between freestone and clingstone peaches. Recent studies have shown that some of the differences in structure reported for these New Jersey peaches, such as a physical breakdown of cell walls in ripe Elbertas, do not apply to California grown varieties *(19)*.

Differences in cell size and, consequently, in cell number found in the present studies between peaches of high and low nitrogen status reflect two interrelated influences of nitrogen — cell production and delayed maturation. Production of more cells could only take place during the embryonic or formative-meristematic stage, including the cell division phase of fruit growth as defined by Addoms *et al. (1)*. Soon after the cell division phases of growth cease in the fruit flesh, the pits are virtually full size and begin to harden; the young flesh then is only about ¼ inch deep and the young fruit is lenticular and pointed, as may be seen in most varieties a month to 6 weeks after fruit set. Subsequent flesh growth to maturity is almost entirely by cell enlargement since peaches do not develop large intercellular spaces.

The production of larger numbers of smaller cells in the high than in the low nitrogen conditions with these Washington State peaches may conceivably relate also to harvest status and the growing season of that

area. Although, as previously cited, increased available nitrogen has been found to result in larger leaves and larger cells, it should be realized that fruit tissues differ physiologically from vegetative tissues by conditions associated with ripening and senescence. The high nitrogen trees of these Washington State peaches were larger leaved and of characteristically vigorous growth by comparison with the low nitrogen trees. However, leaves reach their full size on a peach tree relatively early in the fruit growth season. It would appear that the high nitrogen fruit may be potentially capable of becoming much larger in both cell size and over-all fruit size than the low nitrogen fruit, depending upon season and time left on the tree. On the other hand, it may be that these fruits, after reaching a certain physiological maturity, ripen irrespective of cell size.

It is significant in the present and related investigations *(5, 13)* that differences in structure and texture can be manipulated through nitrogen application during fruit growth. That variations in the texture of fruit and vegetables are influenced by such factors as composition of their pectins and other cell wall substances is well known. Practicalities of firming certain commercially processed fruits and vegetables with calcium likewise seem to be well recognized. The extent to which control of other quality requirements may be possible through cultivation practices remains to be learned. The edible parts of fruits, for example, vary as to tissue origins. Some, as peaches, are formed directly from the ovary of the flower; others, as apples and pears, are formed mainly from accessory floral parts or appendages bearing closer relationship to vegetative organs *(6)*. Responses of different fruits to such practices as nitrogen application may therefore differ accordingly. Different types of fertilizers and local growing conditions likewise are important *(8)*. These situations indicate need for further fundamental investigations.

SUMMARY

Washington State Elberta peaches harvested for three seasons from trees growing in soil management plots of different nitrogen status have been found to differ in texture and structure. Fruit of high nitrogen nutrition had more numerous, smaller flesh parenchyma cells than that grown under conditions of low nitrogen availability. In peaches of each nitrogen status cell numbers were found more important than cell size in determining fruit size. These cellular distinctions between high and low nitrogen peaches serve to explain differences in texture appearance of the canned fruit. In canning qualities, the high nitrogen fruit remained firm and finely textured while the low nitrogen fruit was coarse, stringy, and often ragged in appearance. The role of available nitrogen in producing different cellular effects in fruit and vegetable tissues is discussed.

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SCORING FOR THREE COMPONENTS OF TENDERNESS TO CHARACTERIZE DIFFERENCES AMONG BEEF STEAKS[a, b]

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Because tenderness is an important attribute of the eating quality of meat, a reliable method of controlling it is needed. But control must await a reliable method of measuring tenderness and perhaps further insight into the chemical and physical causes. Meanwhile, however, systematic efforts to tabulate the various kinds and degrees of tenderness should contribute to the identification of chemical or physical changes associated with tenderness.

In the past, members of a judging panel have been asked to record only one score for tenderness of beef. When such scoring is done on only one cut cooked by only one standardized method, scoring for tenderness is relatively simple. But when judges are presented with meat from different parts of the carcass cooked in several different ways, they must relate a variety of sensations to tenderness. Then, scoring may be exceedingly complex and the scores difficult to interpret. A few illustrations may make this point clear. Cover *et al. (4, 6)* have suggested that judges' scores for tenderness of rare steaks may be influenced by their softness. Swallowing these soft juicy morsels may take place before the tenderness of either the muscle fibers or the connective tissue has been assessed. But if rare steaks are chewed thoroughly, coarse connective tissue will be very evident in the soft juicy morsels from round steaks, though probably not in those from loin steaks. As another illustration, the muscle fibers of braised bottom round steaks may crumble easily into tiny fragments and little or no connective tissue may be felt. With braised loin steaks, however, the meat may be stringy because little connective tissue is present and the muscle fibers do not crumble readily. Such differences in tenderness characteristics are difficult for a judge to report in a single score for tenderness. Yet, if chemical causes of tenderness or toughness are to be investigated, precise descriptions of tenderness changes during cooking are imperative. It is especially important to separate toughness caused by connective tissue from that caused by muscle fibers. Partitioning tenderness into a component from muscle fibers and one from connective tissue was attempted by Lehman *(9)* as long ago as 1907.

Various mechanical devices for measuring tenderness objectively have been constructed *(1, 3, 7, 8, 10, 11, 13, 14, 15, 16, 17)*. Schultz *(12)* did a very good job of describing them. But none of these devices measures all aspects of tenderness.

It seemed possible that intelligent personnel might be trained to discriminate among the different tenderness sensations. Their scores would

[a] The meat used was from animals raised at Substation 23, McGregor, Texas under a project which is cooperative with the U. S. Department of Agriculture and is a part of S-10 Southern Regional Beef Cattle Breeding Project.

[b] The author wishes to acknowledge the assistance of R. L. Smith, Data Processing Center, for making the statistical calculations.

give information not possible to obtain from present mechanical devices. The results of such an attempt are herewith presented.

EXPERIMENTAL

Materials. The meat used came from 55 yearling steers of about the same age, produced in 1955–6 at Substation No. 23 of the Texas Agricultural Experiment Station. They had been fed the same ration under similar conditions. Four carcasses graded U. S. Choice, 24 U. S. Good, 26 U. S. Standard, and one U. S. Utility. These steers were Santa Gertrudis, Herefords, Brahmans, and various crosses between Herefords and Brahmans. The relationship of tenderness of these steers to inheritance has been reported by Cartwright, Butler and Cover *(2)* and the relationship of carcass grade, separable fat and ether extract to tenderness by Cover, King and Butler *(5)*. (Two other publications are in preparation, one on collagen content, and one on the effect of carcass grade and fatness on the tenderness obtained after cooking by moist and dry heat methods.)

The steers were slaughtered in the meats laboratory of the Animal Husbandry Department. After storage for 7 days, each carcass was divided into the standard wholesale cuts and then into 1-inch steaks taken from short loin and bottom round. Two steaks from the anterior end of the loin and 2 from the dorsal end of the bottom round were obtained from each side of each carcass for these tests. Loin steaks were trimmed to include the *longissimus dorsi* muscle and adhering fat but no bone or other muscles. Bottom round steaks were trimmed to include the *biceps femoris* muscle and adhering fat but no bone or other muscles. The steaks were wrapped individually, frozen at minus 20° F. and stored at 0° F. until tests could be made.

The 4 steaks from each cut were cooked—two by a "moist heat method" (braising) and two by a "dry heat method" (oven-broiling). Within each of these methods, the meat was cooked to two internal temperatures which were selected to provide extremes of doneness by each method of cooking.[e] Thermometers were inserted into the frozen steaks when the temperature was about minus 2° C. and the cooking was started as nearly as possible at 2° C. (35.6° F.).

Oven-broiling. Steaks were broiled in well-ventilated gas ovens with the oven doors closed. Only one steak was cooked in each oven. Both oven and meat thermometers were read through the glass oven door. The oven, with the broiler pan in place, was regulated at 175° C. (347° F.) before broiling was begun. Because the broiler pan was lined with aluminum foil and an open wire rack was used, the steak browned on the under side without turning. The rare steaks were removed at an internal temperature of 61° C. (141.8° F.) and the well-done ones at 80° C. (176° F.).

Braising. The braised steaks were browned and then cooked in steam. A heavy pot was preheated to 475° F. (246.1° C.) in a standardized manner over a gas flame. One tablespoon of cooking oil was added and the steak browned for one minute on each side. After browning, a rack was placed under the steak, hot water was added to a short distance below the rack, and the heavy lid was adjusted. The liquid was brought to boiling quickly, and the fire adjusted to maintain a steady flow of steam through the opening in the lid around the meat thermometer. Steak temperatures were recorded at 1-minute intervals. Only one steak was cooked in each pot. Half of the braised steaks were removed at an internal temperature of 85° C. For the others, the cooking was continued to an internal temperature of 100° C. and held there for 25 minutes. The steaks which were removed at 85° C. were under-done. The meat was slightly pink and the juice had a pink tinge. The steaks which were cooked to an internal temperature of 100° C. and held there for 25 minutes were very well-done. They were used to simulate the conditions in many homes where braising time may be from 45 to 60 minutes.

Sampling. Cores for shears and samples for the judges were cut as described by Cover and Smith *(6)*. Steaks 1 and 2 from both sides of the same animal provided meat for the 4 conditions of cooking each cut. This made a total of 4 loin and 4 bottom round steaks for one judging session. The composition of the judging committee was uniform

[e] Meat from a few animals had a slight pink color after broiling to 80° C. While pink juice did not run out of the meat or collect on the plate, a tiny amount of pink juice could be pressed out by squeezing with the fingers. The cause of this unusual condition is unknown but it is distinctly an animal variation.

for all of the animals included in this report. Two more animals were available but were excluded because a judge was absent unexpectedly.

Judges' scores. Scores for juiciness were obtained as in previous studies (4, 6) but the tenderness scores were obtained in a different manner. They were broken down into softness and friability of muscle fibers and tenderness of connective tissue. Softness was rated according to the sensations from tongue and cheek and by the ease with which the teeth sank into the meat at the first bite. Friability was rated on the ease with which the muscle fibers broke—whether they tended to be crumbly or rubbery. Tenderness of connective tissue was rated by the quantity of connective tissue and its resistance to chewing. The adjectives used in scoring and their associated weightings are given in Table 1. When these scores were averaged, the numerical intervals were assigned as in Table 2.

Statistical treatment of the data. Analyses of variance were calculated using the outlines given in Table 3. Coefficients of correlation were calculated for certain pairs of variables.

TABLE 1

Adjectives used in scoring meat and associated weightings (1956 data)

Weighting of adjectives	Adjectives used in scoring			
	Juiciness	Softness	Friability of muscle fibers	Tenderness of residual connective tissue
10	Extremely juicy	Extremely soft	Extremely friable	No C. T. left
9	Very juicy	Very soft	Very friable	Tiny amt. of soft C. T.
8				
7	Juicy	Soft	Friable	Small amt. of fairly soft C. T.
6				
5	Neither juicy nor dry	Neither hard nor soft	Moderately friable	Small amt. of firm C. T.
4				
3	Dry	Hard	Slightly friable	Medium amt. of firm C. T.
2				
1	Very dry	Very hard	Very slightly friable	Large amt. of hard C. T.
0	Too dry to swallow	Too hard to chew	Not friable	Residue very hard

RESULTS AND DISCUSSION

Weight of steak, weight loss and cooking time are given in Table 4. They follow patterns similar to those of previous studies in this laboratory *(4, 6)*.

Comparisons by stages of doneness. Broiled steaks of both muscles, when rare, scored very juicy, soft and moderately friable, but when well-done, they were "neither juicy nor dry," "neither hard nor soft" and were "friable" (Table 5). Thus, as doneness increased, these broiled steaks became less juicy and less soft but more friable. The connective tissue in *longissimus* was so tender that there was little or no possibility for improvement with doneness, but in *biceps* the rare broiled steaks had a considerable

TABLE 2

Numerical intervals associated with adjectives in judges' scores (1956 data)

Numerical intervals	Adjectives used in scoring			
	Juiciness	Softness	Friability of muscle fibers	Tenderness of residual connective tissue
9.5 to 10.0	Extremely juicy	Extremely soft	Extremely friable	No C. T. left
8.0 to 9.4	Very juicy	Very soft	Very friable	Tiny amt. of soft C. T.
6.0 to 7.9	Juicy	Soft	Friable	Small amt. of fairly soft C. T.
4.0 to 5.9	Neither juicy nor dry	Neither hard nor soft	Moderately friable	Small amt. of firm C. T.
2.0 to 3.9	Dry	Hard	Slightly friable	Medium amt. of firm C. T.
0.5 to 1.9	Very dry	Very hard	Very slightly friable	Large amt. of firm C. T.
0 to 0.4	Too dry to swallow	Too hard to chew	Not friable	Residue very hard

TABLE 3

Outline of analyses of variance

Source of variation	Degrees of freedom	Error terms
Analysis I.		
Cuts	1	Animals
Methods within cuts	6	Animals
Doneness within broiled loin	(1)	Animals
Doneness within braised loin	(1)	Animals
Broiled vs. braised within loin	(1)	Animals
Doneness within broiled bottom round	(1)	Animals
Doneness within braised bottom round	(1)	Animals
Broiled vs. braised within bottom round	(1)	Animals
Animals within cuts and methods	432	
Total	439	
Analysis II.		
Methods	3	Animals
Cuts within methods	4	Animals
Loin vs. bottom round within 61° C.	(1)	Animals
Loin vs. bottom round within 80° C.	(1)	Animals
Loin vs. bottom round within 85° C.	(1)	Animals
Loin vs. bottom round within 100° C. +	(1)	Animals
Animals within cuts and methods	432	
Total	439	
Analysis III.		
Cuts	1	Animals
Methods within cuts	2	Animals
80° C. vs. 100° C. + within loin	(1)	Animals
80° C. vs. 100° C. + within bottom round	(1)	Animals
Animals within cuts and methods	216	
Total	219	

TABLE 4

Weights, weight losses and cooking times, averages of one steak from each of 55 animals

Cuts, methods of cooking, and final steak temperatures[1]			Weight of steak (g.)	Cooking time (min.)	Weight loss (%)
Comparison by temperatures within methods and cuts					
Loin (*L. dorsi*)	Broil	61° C.	198	24	13
		80° C.	208n. s.	56***	31***
	Braise	85° C.	196	11	23
		100° C.+	210*	46***	36***
Bottom round (*B. femoris*)	Broil	61° C.	279	29	13
		80° C.	275n. s.	70***	37***
	Braise	85° C.	287	15	24
		100° C.+	277n. s.	50***	39***
Comparison by cuts within temperatures and methods					
Broil	61° C.	Loin	198	24	13
		BR	279***	29**	13n. s.
	80° C.	Loin	208	56	31
		BR	275***	70***	37***
Braise	85° C.	Loin	196	11	23
		BR	287***	15*	24***
	100° C.+	Loin	210	46	36
		BR	277***	50*	39***
Comparison by methods within cuts cooked well-done					
Loin	Broil	80° C.	208	56	31
	Braise	100° C.+	210n. s.	46***	36***
Bottom round	Broil	80° C.	275	70	37
	Braise	100° C.+	277n. s.	50***	39***

[1] The cuts were loin and bottom round (BR). The methods of cooking were broiling to 61° C. and 80° C. and braising to 85° C. and to 100° C. plus 25 minutes.

n.s. = significance above 5% level.
* = at 5% level.
** = at 1% level.
*** = at 0.1% level.

amount of firm connective tissue which increased significantly in tenderness as doneness increased. Shear force values increased with increased doneness in broiled loin steaks (the meat became tougher) but the broiled bottom round steaks showed little change with doneness.

The braised steaks of both muscles decreased in juiciness as doneness increased. Scores for softness, friability and tenderness of connective tissue did not seem to be affected by doneness in braised loin steaks but in bottom round steaks the ones which were braised longer were softer, more friable and the connective tissue was more tender. Shear force values of braised steaks increased with doneness in loin (the meat became tougher) but decreased with doneness in bottom round (the meat became more tender). The trend of shear force values in braised loin was not explained by the scores for softness, friability or tenderness of connective tissue. In braised

bottom round, however, the trend of shearing tenderness with doneness was similar to that of scores for softness, friability and tenderness of connective tissue.

Comparisons between the two muscles. Only small differences were noted in juiciness between the two muscles when they were cooked alike (Table 5).

Softness scores indicated that the two muscles were about equally soft when broiled rare (61° C.); about equally firm when broiled well-done (80° C.) and when braised to 85° C.; but *longissimus* was firmer than *biceps* when braised to 100° C. +.

TABLE 5

Juiciness scores and tenderness data, averages of one steak from each of 55 animals

Cuts, methods of cooking and final steak temperatures			Scores				Shear force value in pounds[2]
			Juiciness[1]	Softness[1]	Friability of muscle fibers[1]	Tenderness of connective tissue[1]	
Comparison by stages of doneness							
Loin (*L. dorsi*)	Broil	61° C.	8.5	7.5	5.3	9.2	7.4
		80° C.	5.9***	5.2***	6.3***	9.6n. s.	11.8***
	Braise	85° C.	5.5	4.9	6.2	9.5	9.7
		100° C.+	3.0***	4.7n. s.	6.5n. s.	9.3n. s.	12.5***
Bottom round (*B. femoris*)	Broil	61° C.	8.8	7.5	5.6	3.1	8.9
		80° C.	5.0***	5.2***	7.1***	5.5***	9.0n. s.
	Braise	85° C.	5.5	4.7	6.7	5.0	9.0
		100° C.+	3.3***	5.9***	8.0***	9.3***	7.4***
Comparison between the two cuts							
Broil	61° C.	Loin	8.5	7.5	5.3	9.2	7.4
		BR	8.8n. s.	7.6n. s.	5.6n. s.	3.1***	8.9***
	80° C.	Loin	5.9	5.2	6.3	9.6	11.8
		BR	5.0***	5.2n. s.	7.1**	5.5***	9.0***
Braise	85° C.	Loin	5.5	4.9	6.2	9.5	9.7
		BR	5.5n. s.	4.7n. s.	6.7n. s.	5.0***	9.0n. s.
	100° C.+	Loin	3.0	4.7	6.5	9.4	12.5
		BR	3.3**	5.9***	8.0***	9.3n. s.	7.4***
Comparison by methods within cuts cooked well-done							
Loin	Broil	80° C.	5.9	5.2	6.3	9.6	11.8
	Braise	100° C.+	3.0***	4.7*	6.5n. s.	9.4n. s.	12.5n. s.
Bottom round	Broil	80° C.	5.0	5.2	7.1	5.5	9.0
	Braise	100° C.+	3.3***	5.9***	8.0**	9.3***	7.4**

[1] The scores of the 4 judges were averaged to obtain the steak score. Then the 55 steak scores were averaged. Possible scores were from 0 (lowest) to 10 (highest).

[2] The shear force values of 4 cores were averaged to obtain the shear value for the steak. Then the shear values for the 55 steaks were averaged.

n.s. = significance above 5% level.
* = at 5% level.
** = at 1% level.
*** = at 0.1% level.

Friability scores indicated that the 2 muscles were about equally friable when broiled to 61° C. and also when braised to 85° C.; but *biceps* was more friable than *longissimus* when broiled to 80° C. and when braised to 100° C. +. Can this mean that the proteins of the muscle fibers in the 2 muscles react differently to heat?

Scores for tenderness of connective tissue indicated that only in steaks braised to 100° C. + was the connective tissue in *biceps* as tender as that in *longissimus*. In all other temperature-method comparisons the connective tissue in *biceps* was much tougher than that in *longissimus*.

Shear force values indicated that: *longissimus* was slightly but significantly more tender than *biceps* when broiled rare (61° C.); there was no significant difference between the 2 muscles when they were braised to 85° C.; but *biceps* were significantly more tender than *longissimus* when they were broiled to 80° C. and braised to 100° C. +.

Correlations. When the 4 conditions of cooking within each cut were combined for these 55 animals, there were 220 loin steaks and 220 bottom round steaks. The range within a muscle therefore was very wide for weight loss, shear force values and scores for juiciness, softness, friability of muscle fibers and tenderness of connective tissue (Table 6). Correlation coefficients were calculated for all possible pairs of these variables. For convenience these coefficients are arranged into 5 groups in Table 7.

In the juiciness group, juiciness score was more highly correlated with weight loss than with any of the other variables in Table 7 and the coefficients were similar for the 2 muscles. The coefficients for juiciness score versus softness score also were similar for both muscles. The juiciest steaks tended to be those which lost the least weight and those which were the softest. These results are in line with traditional ideas.

The coefficients for juiciness score versus each of the other variables in the first group of Table 7 were considerably different for the 2 muscles. The drier steaks tended to be more friable but this association appeared to be closer in bottom round than in loin steaks. This finding brings into question some of the older ideas about toughening of meat proteins by heat.

The juicier steaks tended to have tougher connective tissue. This relationship was low and not significant in loin steaks, where the connective tissue was very tender by all methods of cooking, but it was rather high and significant for bottom round steaks, where the connective tissue was tough at 61° C. and very tender at 100° C. +. The traditional concept of tendering of heavy connective tissue in round steaks on longer cooking is involved here.

TABLE 6

Ranges for 6 variables in loin and bottom round steaks

Variables	No. of steaks averaged[1]	Ranges	
		Loin	Bottom round
Weight loss (%)	220	9.0–40.3	10.7–45.3
Juiciness score	220	1.5–10.0	2.0– 9.8
Softness score	220	2.0– 9.0	3.5– 8.5
Friability score	220	1.3– 9.8	4.0– 9.8
Connective tissue score	220	5.3–10.0	1.5–10.0
Shear force value (lb.)	220	3.4–20.7	5.2–11.6

[1] One steak by each of 4 methods of cooking x 55 animals = 220 steaks.

TABLE 7

Correlation coefficients calculated within loin steaks and within bottom round steaks for each pair of variables

Variables	No. of steaks in each group[1]	Correlation coefficients	
		Loin (*L. dorsi*)	Bottom round (*B. femoris*)
Juiciness score versus:			
Weight loss	220	−.848***	−.896***
Softness score	220	.612***	.593***
Friability score	220	−.210***	−.673***
Connective tissue score	220	−.118n. s.	−.764***
Shear force value	220	−.465***	.323***
Softness score versus:			
Weight loss	220	−.604***	−.498***
Friability score	220	.406***	−.209**
Connective tissue score	220	.150*	−.191**
Shear force value	220	−.764***	−.176*
Friability score versus:			
Weight loss	220	.153*	.685***
Connective tissue score	220	.521***	.690***
Shear force value	220	−.512***	−.379***
C. T. score versus:			
Weight loss	220	−.111n. s.	.743***
Shear force value	220	−.233*	−.489***
Shear force value versus:			
Weight loss	220	.595***	−.270**

[1] One steak by each of 4 methods of cooking x 55 animals = 220 steaks.

n.s. = significance above 5% level.
* = at 5% level.
** = at 1% level.
*** = at 0.1% level.

The relationship of juiciness to shear force value was highly significant in both muscles but in loin the juicier steaks had the lower shear force value (more tender) while in bottom round steaks the juicier steaks had the higher shear force value (tougher).

Thus, when we attempt to untangle juiciness score from tenderness we find that *longissimus* and *biceps* behave similarly for juiciness versus softness but the 2 muscles behave differently for juiciness versus shear force, friability of muscle fibers, and tenderness of connective tissue. This suggests that there are fundamental differences between the 2 muscles, not only in connective tissue but in muscle fibers as well. The possibility that chemical differences are responsible should lead to further work.

Some of the variability in softness score was accounted for by weight loss, with the softer meat having the lower weight loss and with both cuts showing fairly similar trends (Table 7). With all other variables in this group, however, the differences between the 2 muscles were rather marked.

The softness score was significantly correlated with friability score in each muscle but in loin steaks the softer meat tended to be more friable while in bottom round steaks the harder meat tended to be more friable. The coefficients for softness versus tenderness of connective tissue were low but significant and were in opposite directions for the two cuts.

Softness score and shear force value of loin steaks were the most closely correlated of any comparisons within this group—with the softer meat

tending to be associated with the lower shear (tender meat). Yet, in bottom round steaks the coefficient is one of the lowest.

Greater friability seemed to be associated with greater loss in weight but the coefficient was very low in loin while for bottom round it was one of the highest within this small group. Greater friability was associated with greater tenderness of connective tissue in both cuts. Greater friability was correlated with lower shear force value (more tender meat) but the coefficient was somewhat higher for loin than for bottom round.

Tenderness of connective tissue was not significantly associated with weight loss in loin but the coefficient was moderately high for bottom round, with the tenderer connective tissue associated with the greater weight loss. The coefficient for tenderness of connective tissue versus shear force was low for loin and somewhat higher for bottom round, but for both cuts it indicated that the connective tissue tended to be more tender with the lower shear force value. A correlation between tenderness of connective tissue versus collagen content is of considerable interest. This relationship will be reported in another publication. The effect of carcass grade and fatness on tenderness of connective tissue after each of these methods of cooking also will be reported in another publication.

Lower shear force value (more tender meat) tended to be associated with lower weight loss in loin but with higher weight loss in bottom round.

SUMMARY AND CONCLUSIONS

Four steaks from each of 2 cuts, loin and bottom round, from each of 55 animals were used. Each of the 4 steaks from a cut were cooked differently. Two were oven-broiled (one to 61° C., rare; and one to 80° C., well-done) and 2 were braised (one to 85° C., medium-rare; and one to 100° C. and held there for 25 minutes, very well-done). Shear force values and judges' scores for juiciness were obtained in the usual manner but instead of a single score for tenderness, scores were obtained for softness, friability of muscle fibers and tenderness of connective tissue.

The scores for juiciness were remarkably similar to those of the previous study *(4)*.

Scoring for tenderness of connective tissue made it possible to confirm some of the traditional beliefs about the tenderness of connective tissue in these two cuts. The tenderness of connective tissue in bottom round was scored low (3.1) when broiled rare; medium when broiled well-done (5.5) and braised medium-rare (5.0); but very tender (9.3) when braised very well-done. Thus the connective tissue in bottom round was made tender by braising to a high internal temperature (100° C.) and holding there for 25 minutes. In contrast, the connective tissue in loin was so tender that no significant effect was obtained from the different methods of cooking.

Scores for softness were highest in the steaks broiled rare to 61° C. Broiling well-done (80° C.) and braising (85° C. and 100° C. +) seemed to harden the muscle fibers in both loin and bottom round.

Scores for friability indicated that the muscle fibers in loin steaks broke apart most readily when broiled rare and were less friable after the other 3 conditions of cooking. In bottom round steaks however, the most thoroughly cooked steaks had the most friable fibers. The contrast between the 2 cuts

in friability of the muscle fibers was marked. The reason for this finding is not readily apparent. It offers many possibilities for further work.

Judges apparently were able to distinguish successfully between the following components of tenderness: softness, friability and the tenderness of connective tissue. Although further research may indicate that additional components are important, the method of partitioning tenderness among several components seems to offer increased opportunities for relating chemical and physical changes to certain kinds and degrees of tenderness.

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ODOR DIFFERENCE TEST WITH APPLICATIONS TO CONSUMER PREFERENCES

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It has been shown in two previous papers *(1, 2)* that the ability to detect differences between sucrose and tartaric solutions varies tremendously from one individual to another. In this paper we present two odor difference experiments which show that olfactory sensitivity varies so greatly among people that differences always detected by some are never detected by others, with graduations between. Since these differences exist it is necessary to define "consumer preference" in terms of the consumers. The usual case seems to be that many consumers cannot detect at all the differences that are critical for others. The realization of this fact could well lead to a drastic reorganization of processing, advertising, and marketing of foods based upon extensive surveys of the olfactory and taste abilities of the potential consumers.

As a matter of emphasis we discuss a hypothetical example of a possible "consumer" group suggested by our actual experiment.

ODOR DIFFERENCE TESTS

These experiments were conducted in the spring of 1958 with phenylethyl alcohol. The 14 testers were students and staff members at the University. The general plan of the experiments was similar to that reported by Wenzel *(4)*.

The equipment to deliver a known amount of odor material mixed with a known amount of clean air was constructed in the following manner. Clean outside air was pumped through the system by a Rexair (Rainbow Model D) vacuum cleaner. The air was precooled and washed before it entered the system. This was accomplished by the addition of an ice-water mixture to the dust bowl of this particular type of vacuum cleaner. The air then passed through a charcoal filter to a large Fischer-Porter triflot flow meter (capacity 21 ft^3 per minute) which was used to regulate the flow of the air to 13 ft^3 per minute to the odor hood. Part of the clean filtered air was by-passed and bubbled through a flask containing the pure test liquid, phenylethyl alcohol (PEA). A pair of small flow meters (covering a range of 3.0 — 12,000 cc. per minute) were used to regulate the flow of known amounts of odor-saturated vapor back into the main air stream.

A Pyrex line, three feet long and 1½ inches in diameter, was used for mixing the odor-saturated air with the main air stream. The gases then entered the odor hood. The hood was a rectangular structure made of plexiglass. The dimensions were 17½ inches high, 17½ long, and 13½ inches wide. The entrance of the gases was to the side of the subject's head and a few inches over the top of his head. The outlet for the gases was a sliding plexiglass door in the bottom of the hood with an opening large enough to comfortably allow the entrance of the subject's head. Glass or metal was used wherever it was possible. All rubber or other odor giving material was covered with polyethylene. The small flexible joints were of tygon tubing and larger joints were of spun glass flexible hose.

The standard flow was 0.0045 mg. of PEA per liter of air. The lower flows were 0.0020, 0.0029, and 0.0040 mg. of PEA per liter of air. The higher values were 0.0055, 0.0070, and 0.0084 mg. PEA per liter of air. We shall number these latter concentrations 1, 2, 3, 4, 5, 6 from low to high and refer to them by number.

Preliminary tests were run on each person. The concentrations were very low at first and then were gradually increased until the testers identified the odor without difficulty.

In the first experiment unknowns were given each day for three 5-day weeks so that

each concentration was compared with the standard (0.0045 mg. of PEA/liter of air) 5 times each week or 15 times in all. These data are presented in Table 1. The concentrations were randomized for each day during the first week and by weeks for the last 2 weeks. The tester put his head into the chamber; the standard was turned on; the machine ran 10 seconds; the standard was turned off 10 seconds; the unknown was turned on 10 seconds; and then the tester was asked to respond weaker or stronger. A bell rang at the beginning and end of each 10 seconds.

TABLE 1

Number of misses out of 15 trials when an unknown is compared with the standard which is administered immediately prior to the unknown

Tester	Unknown						Total	Percent
	1	2	3	4	5	6		
4	0	0	4	3	0	0	7	7.8
3	0	0	2	4	1	0	7	7.8
1	0	0	3	3	2	0	8	8.9
11	1	1	1	1	1	0	5	5.6
7	0	0	2	4	2	2	10	11.1
10	2	1	3	5	0	0	11	12.2
2	1	2	4	6	1	0	14	15.6
14	1	1	1	7	4	1	15	16.7
6	0	4	2	4	1	4	15	16.7
8	2	2	3	6	2	2	17	18.9
12	4	5	6	3	0	0	18	20.0
13	4	4	4	8	3	0	23	25.6
5	2	3	3	8	6	2	24	26.7
9	10	7	8	8	4	5	42	46.7
Total	27	30	46	70	27	16	216	
Percent	12.9	14.3	21.9	33.3	12.9	7.6	17.1	

In the second experiment each of the 6 unknowns were given 7 times. Nine unknowns were given each test period with the standard before each group of three. The order in which the unknowns were given was randomized over the entire test period. Forty-two trials were made for each tester. The data are given in Table 2. The standard and unknowns were administered in the same ways as in the first experiment.

ANALYSIS AND DISCUSSION OF THE EXPERIMENTS

In Table 1 if the number missed is three or less we reject the null hypothesis of no discrimination at less than the 5% level [one-tailed test *(3)*]. On the basis of the total number of misses all testers show some ability to detect differences from the standard except tester number 9. Further, if atttention is paid to the patterns of the misses additional inferences are made plausible. Some testers are more sensitive to differences below the standard than above it and vice versa. Some testers have sharper cut-offs above or below or both than others. There is great variation in the ability of testers to detect odor differences with respect to a standard. In general the unknowns differing the most from the standard were missed less frequently than the unknowns closer to the standard. The procedures are not sensitive enough and the number of testers is too few to indicate too clearly the relationship between concentration and number of misses. Also, the results cannot be extrapolated to larger populations without question. However, we note, that if the logarithms of the absolute values of the

TABLE 2

Number of misses out of 7 trials when a series of three unknowns is compared with the standard which is administered immediately prior to the series of three

Tester	Unknown						Total	Percent
	1	2	3	4	5	6		
4	1	1	3	3	0	0	8	19.0
3	0	0	2	1	0	0	3	7.1
1	1	1	3	1	2	1	9	21.4
11	1	0	0	4	2	0	7	16.7
7	0	1	2	3	2	0	8	19.0
10	0	0	2	3	0	0	5	11.9
2	0	0	1	2	0	0	3	7.1
14	0	0	1	3	1	3	8	19.0
6	0	2	0	1	1	1	5	11.9
8	0	3	2	3	1	2	11	26.2
12	1	1	2	2	0	0	6	14.3
13	5	2	3	2	0	1	13	31.0
5	1	1	2	4	3	1	12	28.6
9	2	3	1	3	3	2	14	33.3
Total	12	15	24	35	15	11	112	
Percent	12.2	15.3	24.5	35.7	15.3	11.2	19.0	

differences between the unknown and the standard are plotted against the number of misses that the relationship appears to be linear.

The data of Table 2 is not as different from that of Table 1 as might be expected. The number of misses allowable in a cell to reject the null hypothesis at less than the 5% level is 0 [one-tailed test *(3)*]. However, because of patterns the regions of detectability can be extended. In fact all testers, even number 9, show significant ability to detect differences correctly. The total number of misses is not significantly different from the total number of misses in the first experiment [$\chi^2 = 1.00$ with 1 d. f. two-tailed test *(3)*]. The total numbers of misses for each unknown are very similar to the corresponding numbers for the data of Table 1. As a rough method of comparing the results of the 2 experiments we computed the correlation coefficient for the total percents of misses for the 14 testers. The correlation coefficient turned out to be 0.68 indicating considerable shifting of relative standing of individuals from one experiment to the other. Also the application of chi-square shows differences for individual testers for the 2 experiments.

In general, these data indicate great variation between individuals in their ability to detect odor differences. It appears that the plan of the difference testing experiment does not affect all individuals in the same way.

HYPOTHETICAL EXAMPLE

A study of Table 1 reveals that there might well be olfactory differences which are always detected by ⅓ of a group, detected about ½ the time by another ⅓ of the group, and that ⅓ of the group may have little or no ability to detect the difference. In Table 3 we compare probability distributions for number of misses on a total of 12 trials for each of the three types of testers and an equal mixture of the three types.

Suppose that we agree to accept the hypothesis of ability to detect the difference if the number of misses is two or less out of 12. Then for those testers who can always detect the difference (column 1, Table 3) we will always accept the hypothesis of a difference because there will always be 12 zero misses. For those testers without detecting ability (column 2, Table 3) we will accept the hypothesis of a difference about 2 times in one hundred. For those testers that can detect a difference about one-half the time (column 3, Table 3) we will accept the hypothesis of a difference 74 times out of 100. For an equal mixture of the 3 types of testers (column 4, Table 3) we will accept the hypothesis of a difference 58 times out of 100.

TABLE 3

Probability distributions for: (1) tester that always detects a difference, (2) tester with no detecting ability, (3) tester that can detect a difference one-half the time, and (4) a mixture of equal proportions of the first three types of testers. It is assumed that there are 12 testers in the first three cases and 4 of each type in the fourth case

Number of misses	Probabilities			
	1	2	3	4
0	1.0000	.0002	.1467	.3465
1	—	.0029	.3012	.0703
2	—	.0161	.2924	.1582
3	—	.0537	.1720	.1953
4	—	.1208	.0683	.1442
5	—	.1934	.0193	.0651
6	—	.2256	.0040	.0176
7	—	.1934	.0006	.0026
8	—	.1208	.0001	.0002
9	—	.0537	—	—
10	—	.0161	—	—
11	—	.0029	—	—
12	—	.0002	—	—

It is obvious that a processing and marketing procedure that maximizes profits must consider the costs of making changes in a product. An expensive change that can be detected by only a few consumers is not likely to be worthwhile. In order to determine what proportion of the consumers can distinguish stated differences it is necessary to conduct extensive programs under closely controlled conditions. It is possible that sensitive people can eventually be identified by easily observed characteristics if enough data are accumulated bearing on this problem.

SUMMARY

Two experiments on detecting odor differences between unknowns of stated strengths and a standard are presented. In the first case the standard was presented just before the unknown and the tester was asked to respond weaker or stronger. In the second experiment the standard was given immediately before a series of three unknowns and again the tester's responses were weaker or stronger.

These experiments indicate great variation between individuals in their ability to detect odor differences and in their response to the different ways

of presenting the samples. The total percents of misses in the two experiments were not significantly different.

An example of a hypothetical situation suggested by the experiments is given in some detail which indicates that the proportions of the consumers that can detect a stated difference with a given probability are very important in designing a market program that will yield maximum profit.

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SOME FACTORS AFFECTING THE DISPERSIBILITY OF WHOLE EGG SOLIDS IN WATER[a]

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Dispersibility[c] has been studied by many investigators as a measure of the quality of whole egg solids. According to Bate-Smith, Brooks and Hawthorne *(1)* solubility gives a measure of the physical deterioration suffered during processing and storage, the rate of decrease of solubility being a function of temperature and storage. Lowe *(3)* stated the chief disadvantage of dehydrated eggs in food preparation is their instability. Loss of solubility is one instability defect which markedly impairs the functional properties of whole egg solids. McBride *(4)* reported the restoration of the functional properties of whole egg solids is closely related to the degree of solubility, and moreover, there are two kinds of insolubility, one which is due to improper processing of the egg powder and another which is attributable to the effect of poor storage conditions. For food items in which product acceptability depends principally on the function of whole egg, the degree of dispersibility achieved in the reconstitution process may be a controlling factor in the quality of the finished product.

In general, research workers have acknowledged the importance of dispersibility in the functional performance of whole egg solids. A comparison of the rehydration procedures reported in the literature indicates very little agreement concerning the temperature of egg solids and water used, the method for blending the solids and water together, and the length of and conditions for the reconstitution period.

The purpose of this study was to compare the effect of water temperature and method of mixing on optimum dispersibility in the hydration of spray-dried whole egg solids. In Series A of this report the effect of five temperatures of water and four methods of mixing were studied.

Forsythe *(2)* suggested a procedure of dry-blending the egg solids with dry ingredients prior to the addition of liquid in the first stage of mixing which might eliminate the formation of hard, gummy beads. To study this phase of the problem, samples were prepared to determine the effect of the presence of dry sucrose on dispersibility in relation to the temperature of water and method used for reconstitution. In Series B four concentrations of sucrose, three water temperatures, and two methods of mixing were used.

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[a] Approved by the Director of the Michigan State Agricultural Experiment Station for publication as Journal Paper No. 2324.

[b] General Foods Fund Fellow (1958-59), Michigan State University.

[c] Although the term *solubility* appears frequently in the literature, *dispersibility* is used in this paper to denote the relationship of solids to water in connection with the rehydration process.

EXPERIMENTAL PROCEDURE

The whole egg solids in Series A and Series B were from a common lot with a known history. Fresh shell eggs were broken out, desugared by means of the enzyme glucose-oxidase with hydrogen peroxide, and dried in a Grey-Jensen type dryer (2-4% moisture) with air outlet temperatures of 74° C. The solids were immediately cooled to 46° C., packed in drums (July 19, 1957), and held at 7° C. After an overnight shipment from the processing plant, the egg solids again were stored at 7° C., hermetically sealed in nitrogen atmosphere in 3-pound tins, shipped to Michigan State University (November 4, 1957) and immediately placed in a 5° C. refrigerator. The egg solids were 6 months old when used in Series A and 8 months old when used in Series B. Distilled water was used for reconstitution of the solids in both series. Fine granulated sucrose from a common lot was used in the preparation of the samples in Series B.

Series A: Rehydration of egg solids in water

The formula for dispersibility tests in this series consisted of 130 g. whole egg solids and 360 ml. water. These proportions compared favorably with the U.S.D.A. standard composition of raw egg, 26% egg solids and 74% water *(5)*. The egg solids were used directly from the refrigerator and at a temperature of 5 to 8° C. just before they were combined with the water.

Five temperatures of water, 13, 21, 29, 37, and 45° C., were selected arbitrarily. Four methods of rehydration were used with each temperature of water. All samples were mechanically blended with a Kitchen Aid mixer, model K3-C, with the whip attachment at first speed. Preliminary tests were carried out to establish a length of mixing time which would blend the solids and water without *complete* dispersion for any combination of water temperature and mixing method.

The mixing procedures for the methods used were as follows. In methods 1, 2, and 3, the water was added to the egg solids whereas in method 4 the solids were added to the water. The 4 blending periods remained constant for all methods and were 30, 15, 15, and 15 sec. respectively. The mixing bowl and the beater were scraped down well between blending periods. For *Method 1* the water was added in three portions; 100 ml. initially, 60 ml. after the first blending period, and 200 ml. after the second blending period. In *Method 2* the water was added in 2 portions; 180 ml. initially and 180 ml. after the first blending period. All of the water was added to the egg solids before blending in *Method 3*. In *Method 4* the solids were added to the total amount of water before blending. All rehydrated samples were strained through fine, aluminum wire screening and drained 5 minutes.

Mesh strainers, from 4¼ inch squares of screen with 15 wires per inch, were shaped to fit the mouth of a quart collection jar. A strainer and an aluminum foil pan, 4-inch diameter, were paired, coded, and dried to constant weight to be used in straining each sample and drying the residue.

At the end of the draining period each screen, containing the undispersed solids, was removed carefully from the mouth of the jar, placed on its respective pan, and air-dried to constant weight at 100° C.

A Beckman pH meter, Model H2 with a glass electrode, was used to determine the pH of the screened fluid and of the distilled water used for reconstitution.

As each can of egg powder was opened, 2 samples of approximately 5 g. each were removed and dried to constant weight for moisture determination. In calculating the percentage of residue from the dispersibility tests, an adjustment was made for moisture in the original sample.

Series B: Rehydration of egg solids in water in the presence of sucrose

The Series A basic formula, modified by the addition of 2.54, 4.85, and 7.25% dry sucrose was used for Series B. These amounts were based on 5, 10, and 15% of the sucrose used in standard baked custard as suggested by Lowe *(3)*. A control was also prepared without sucrose.

The same equipment described under Series A was used in the preparation of Series B.

Based on the findings in Series A, 21, 33, and 45° C. were selected as water temperatures for rehydration in Series B.

The whole egg solids and sucrose were dry-blended mechanically for 60 sec. on a Kitchen Aid mixer, model K3-C, with whip attachment at first speed, before the addition of water. The egg solids for the control samples were subjected to the same dry-blending treatment to eliminate differences in dispersion which might be attributed to mechanical agitation. Methods 2 and 3, described under Series A, were used in Series B. Procedures for straining aand drying were the same as in Series A. Methods for determining percentage of undispersed solids duplicated those of Series A.

RESULTS AND DISCUSSION

Series A. Differences in mean per cent residue of whole egg solids rehydrated by 4 methods of mixing and 5 temperatures of water are shown in Table 1. Analysis of variance for these data indicated that differences in dispersibility due to mixing method were significant at the 1% probability level. Differences attributable to water temperature were significant at the 5% probability level.

TABLE 1

Series A: mean percent residue of whole egg solids rehydrated by four methods of mixing and five temperatures of water

Method	Per cent residue						Least significant difference		
	Water °C.							5%	1%
	45°	37°	29°	21°	13°	mean			
No. 1	1.20	1.29	1.72	2.11	2.76	1.82	Temp.		
No. 2	0.55	0.49	0.69	0.50	0.47	0.54	water	0.44	0.59
No. 3	0.48	0.57	0.81	0.75	1.06	0.74	Method	0.39	0.53
No. 4	0.65	0.79	0.48	0.76	1.14	0.76	Cell	0.88	1.18
mean	0.72	0.78	0.93	1.03	1.36				

Evaluation of variations between method averages of mean per cent residue indicated that Method 1 was significantly different [d] from all other methods used. No significant differences were found between averages for Methods 2, 3, and 4.

Comparison between water temperature averages showed that mean per cent residues for samples rehydrated with 45 and 37° C. water were significantly smaller [e] than for samples prepared with 13° C. water. Differences between water temperature averages for mean per cent residue of samples made with 29 and 21° C. water were not significantly different [e] from samples rehydrated with 45, 37, and 13° C. water.

Differences among mean per cent residues for all possible combinations of mixing method and water temperature used in the series were compared. Method No. 1, in combination with each of the 5 water temperatures yielded significantly higher [e] mean per cent residues than those obtained from all other combinations of mixing method and water temperature. Differences between mean per cent residues for samples rehydrated by Methods 2, 3, and 4 in combination with any of the water temperatures studied were not significant.

Within the limitations of this study, Methods 2, 3, and 4 in combination with water temperatures ranging from 45 to 21° C. produced the greatest per cent dispersibility with equally effective results.

[d] Studentized range test: 1% level of probability.
[e] Studentized range test: 5% level of probability.

Determinations of pH for the screened fluid varied from 7.0 to 7.3 and appeared to reflect changes in the pH of the water used to reconstitute the egg solids.

Series B. The mean per cent residue of whole egg solids rehydrated in the presence of dry sucrose are presented in Table 2. Four concentrations of sucrose, 2 methods of mixing, and 3 temperatures of water were used in this series. Analysis of variance for these data indicated that differences in the dispersibility of the egg solids attributable to mixing method or concentration of sucrose were not significant at the 5% level of probability. Differences in mean per cent residue due to the temperature of water used were significant at the 1% level of probability.

TABLE 2

Series B: mean per cent residue of whole egg solids rehydrated in the presence of dry sucrose with four levels of sucrose, two methods of mixing and three temperatures of water

Method	Water °C.	Per cent residue					Least significant difference	
		Sucrose concentration					5%	1%
		0%	2.54%	4.85%	7.25%	mean		
No. 2	45°	0.34	0.26	0.34	0.30	0.310	Temp. water	0.072	0.095
	33°	0.35	0.32	0.53	0.42	0.405			
	21°	0.59	0.50	0.43	0.72	0.560	Sucrose level	0.084	0.111
	mean	0.427	0.360	0.433	0.480				
No. 3	45°	0.34	0.32	0.29	0.35	0.325	Cell	0.203	0.270
	33°	0.33	0.49	0.32	0.36	0.375	Method	0.058	0.077
	21°	0.55	0.55	0.70	0.64	0.610			
	mean	0.407	0.453	0.437	0.450				

Evaluation of differences between water temperature averages showed that mean per cent residues for samples rehydrated with 45 and 33° C. water were not significantly different from each other, although they were significantly smaller [d] than the mean per cent residues of samples prepared with 21° C. water.

To determine the effect on dispersibility for egg solids aerated before the addition of water the mean per cent residue for control samples from Series B, rehydrated with 45 and 21° C. water, were compared with data from Series A for the same mixing methods and water temperatures. Analysis of variance of these data indicated no significant differences attributable to aeration.

In view of the evidence presented above, neither the presence of dry sucrose nor aeration of whole egg solids before the addition of water affected the extent of dispersion achieved in Series B. Whole egg solids evidenced significantly greater dispersibility when rehydrated with 45 and 33° C. water than when rehydrated with 21° C. water.

SUMMARY

The effect of method of mixing, water temperature, and the presence of dry sucrose on optimum dispersibility was investigated in the rehydration of a particular sample of spray-dried whole egg solids of known history.

Methods 2, 3, and 4 in combination with water ranging from 45 to 21° C. produced the greatest per cent dispersibility with equally effective results. The presence of dry sucrose and the aeration of the egg solids before the addition of water had no apparent effect on the dispersion of the whole egg solids.

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A COMPARISON OF THE GELATION PROPERTIES AND PALATABILITY OF SHELL EGGS, FROZEN WHOLE EGGS, AND WHOLE EGG SOLIDS IN STANDARD BAKED CUSTARD[a]

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Whole egg solids, a product long used by the baking industry, are relatively unfamiliar to most food service operators. Since World War II, advances in processing methods and increased availability have brought the product to the attention of this group of potential consumers. As a result, interest in the functional properties and palatability of dried eggs has been stimulated.

Difficulty in reconstituting the egg solids with water has been reported by some food service operators. For products in which acceptability depends chiefly upon the function of whole egg, the extent of dispersion of the egg solids may affect the quality of the finished product. Forsythe *(4)* has suggested incomplete dispersion, resulting in protein dilution, may affect gelation properties adversely in custards and puddings. An investigation of the effect of water temperature, mixing method, and presence of dry sucrose on the dispersibility of whole egg solids was reported earlier in this journal *(9)*.

Although the literature contains numerous reports on improved methods of manufacture and storage, comparatively few investigations of the functional properties of whole egg solids have been published. Jordan and Sisson *(5)*, who compared the use of spray-dried whole eggs with shell eggs in baked custards, found that even though dried egg custards were less firm than those made from shell eggs, they were sufficiently firm to be very desirable in consistency. Bennion, Hawthorne, and Bate-Smith *(2)* noted a definite and rather unpleasant flavor in a number of samples of dried eggs tested in their laboratory that made them unsatisfactory for scrambled eggs or custards. Termohlen, Warren, and Warren *(10)* observed that whole egg solids could be used successfully in baked foods but were much less satisfactory in scrambled eggs, custards, and other products in which egg was the primary ingredient. McBride *(8)* found dried whole egg of good quality produced baked custards comparable to those made with fresh or frozen eggs. Ary and Jordan *(1)*, in a comparison of the effects of five spray-dried whole egg powders, differing in quality, upon the properties of plain butter cakes and baked custards, concluded that plain butter cakes and baked custards comparable in acceptability to similar products made with fresh eggs could be attained with good quality, spray-dried whole egg powders.

The primary objective of this investigation was to compare the gelation properties and palatability of shell eggs, homogenized frozen eggs, blended

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frozen eggs, and reconstituted spray-dried whole egg solids in standard baked custard. Most of the heat-coagulable protein in a custard mixture is furnished by the egg, according to Lowe *(7)*; only 0.75% of the milk protein is heat-coagulable. Inasmuch as firmness of gel is the result of both the coagulation temperature and the time of heating, custards made with the four types of egg were baked to internal temperatures of 86, 88, and 90° C. Because custards contain only a few bland ingredients, evaluation of flavor differences attributable to the type of egg was simplified.

EXPERIMENTAL PROCEDURE

The same lot of glucose-oxidase desugared, spray-dried, whole egg solids were used in the custard and in the earlier study on the effect of water temperature, mixing method and the presence of dry sucrose on the dispersibility of whole egg solids. The preparation and the storage history of the egg solids have been described *(9)*. The egg solids were approximately 10 months old when used in the custards.

The fresh eggs and the eggs used for freezing were obtained from the same flock of White Leghorn hens at the Michigan State University Poultry Farm. The day-old shell eggs were held at 5 to 8° C. and used the following day. One lot of day-old eggs was mechanically blended and frozen; another lot of day-old eggs was homogenized prior to freezing. No additional pretreatments were used. All eggs to be frozen were packed in covered pint plastic containers, quick-frozen at −40° C. for 24 hours, and stored at 0° C. until needed. Both types of frozen eggs were defrosted for 48 hours in a walk-in refrigerator at 5 to 8° C. before they were used for custards.

Homogenized milk, obtained from the Michigan State University Dairy Plant the day before preparation, had been processed 1 to 2 days prior to use. Fine granulated sucrose from a common lot was used in preparing the samples.

On the day of preparation the eggs were treated as follows: fourteen shell eggs were broken out and mechanically blended for 6 min. at first speed using the whip attachment.[c] The defrosted blended and homogenized eggs were removed from the containers but were not given additional mixing. The dried egg solids were prepared by mechanically dry-blending 182 g. solids for 1 min., adding 504 ml. distilled water at 33° C., blending for 60 sec., scraping down well with a rubber spatula, blending 30 sec., scraping down a second time, blending for 30 sec., scraping down, and then blending for 30 sec. more. This rehydration procedure was established from preliminary tests in which the length of mixing time had been increased until the solids appeared to be completely dispersed. All samples of whole egg were brought to room temperature before being combined with the other ingredients.

Formula and preparation procedure. The formula selected for the study, based on that of Lowe *(7)*, consisted of 544 g. whole egg, 283 g. sucrose, and 2765 g. homogenized milk. Percentages of ingredients, based on total weight of the mixture, were whole egg 15.1, sucrose 7.9, and milk 77.0.

The egg and sucrose were blended together 1 min.; the milk was added and the mixture blended 5 min. The custard mix was strained through a medium-fine, wire-mesh household strainer to assure a homogeneous mixture. As quickly as possible, 23 portions of the custard mix were poured into 5-oz. pyrex custard cups to a measured depth of 1¾ in. The cups were placed in a large aluminum baking pan and water (22 to 27° C.) was poured into the pan to a depth equal to that of the custard mix in the cups. Two lead wires from a Brown Electronik Potentiometer High Speed Multiple Point Recorder were suspended from the upper rack of the oven in such a manner that one thermocouple tip was centered in the custard located in the middle position of the baking pan and a second tip was positioned in the water bath just to the right of the custard containing the first thermocouple.

Custards were baked in gas ovens, preheated to 177° C., until the sample carrying the potentiometer lead registered the designated internal temperature. Four replications of

[c] A Kitchen Aid Mixer, Model K 3-C, was used in this blending operation and subsequently.

custard made with each type of egg were baked to end temperatures of 86, 88, and 90° C. Preparation for each day included one replication of each of the 4 types of egg baked to one internal end temperature.

The custards were cooled at room temperature on wire racks for a minimum of 1 hour, covered tightly with heavy aluminum foil, and refrigerated overnight at 5 to 8° C. All samples used in the subjective and objective evaluations were taken directly from the refrigerator as the tests were performed.

Subjective evaluation. From the 23 samples baked for each replication it was possible to select the test samples so that, in the course of 4 replications of one type of egg for a specific end baking temperature, each judge had an equal number of samples from inside bake and outside bake positions as well as front and rear oven baking positions. All samples were coded to minimize identification of egg types by the judges.

The cold custards were scored for quality of crust, inside color, aroma, texture, consistency, and flavor by a panel of six judges. In rating the samples, judges were asked to grade each product numerically from 1 to 7 and to check the most appropriate descriptive terms listed for each characteristic. They were also encouraged to include additional comments not covered on the score card. The panel was requested to evaluate each sample separately without comparison with other samples.

Objective evaluation. Samples used in the objective tests were also randomized in respect to baking position. Two samples, one inside bake and one outside bake position, were used for each objective test. The 2 measurements were recorded and then averaged.

One test used for comparing the firmness of custards was the depth of penetration of the cone of the Precision Penetrometer (Arthur H. Thomas Co.) with 15-sec. penetration for 150-g. load. One set of readings was recorded for samples in which the crust had been loosened from the edge of the cup but otherwise left undisturbed. A second set was taken on additional samples from which the crust had been carefully removed before the penetration tests were made.

Measurements for the calculation of per cent sag were taken for comparing the firmness of custards. In this test an inflexible wire ($\frac{1}{16}$ in. dia.) was inserted into the center of the baked sample before it was removed from the cup. The length of the wire entering the sample was recorded to the nearest $\frac{1}{32}$ in. Next, the sample was loosened carefully, inverted on a flat glass plate, and allowed to stand for 15 min. At the end of the standing period the wire was inserted into the center of the inverted sample and the measurement recorded. Per cent sag was calculated from the difference between readings divided by the original depth determination.

Measurements of resistance to cutting were taken with a Raytheon Curd Tension Meter, model No. 2-505. The crust of the custard was carefully removed and the portion remaining in the cup placed on the platform of the instrument directly under the curd knife. The knife was lowered at a uniform rate by an electrically operated motor. The resistance encountered by the knife in passing through the custard was read directly from the scale of the curd tension meter, calibrated in 5-g. gradations.

For the syneresis test the crust was not removed from the sample. The custard was loosened carefully and inverted on fine wire screening (15 wires per in.) which had been placed over a weighed petri dish. The inverted sample was covered to prevent evaporation and allowed to stand 1 hour. At the end of the inversion period the wire screening, containing the custard, was removed and the weight of the drainage recorded. Per cent drainage was calculated from the ratio of the weight of the drainage to the weight of the sample before inversion.

On the day of preparation, pH determinations were made on duplicate samples of milk, distilled water used for the rehydration of the egg solids, each type of egg used, and each custard mix. On the day following preparation, duplicate solutions of the baked custard for each type of egg used were made by thoroughly blending 10 g. custard with 75 ml. distilled water for each sample tested. Determinations of pH were made on the baked custard solutions and the distilled water used in the preparation of them. Samples for all pH determinations were used at room temperature (22 to 27° C.) and were tested on a Beckman pH meter, model H2 with a glass electrode.

RESULTS AND DISCUSSION

The concentration of whole egg solids used in this study was arbitrarily based on the U.S.D.A. standard composition of raw egg, 26% solids and

74% water. The solids were reconstituted with distilled water prior to the preparation of the custard mix so that all types of eggs used in the experiment would be in a similar liquid state. From the data it appeared that the egg solids did not return to a comparable physical form when reconstituted by the method used in the study. In addition, the investigators realize that the concentration of egg solids may not have been the best selection for use in this test product.

The method of mixing used in the preparation of the custards was also an arbitrary selection. Moreover, the omission of salt and flavoring materials from the formula may have affected the subjective judgments in respect to aroma and flavor. The range of internal end baking temperatures selected for this study included the optimum temperature of gelation for fresh and frozen egg custards but the data seemed to indicate that this range of end temperatures did not include the optimum gelation temperature for dried egg custards with the formula used.

Objective evaluation. The objective tests used for evaluation of the gelation properties of baked custards included three tests for firmness and one test for resistance to cutting.

Mean objective measurements on baked custards prepared from 4 types of whole egg and baked to 3 internal end temperatures are summarized in Table 1. The statistical treatment of the data included an analysis of variance of penetrometer readings (with and without crust), of per cent sag, and of curd tension measurements. The analysis for each test indicated differences in gelation attributable to type of egg and internal baking temperature were significant at the 1% level of probability. The data for each test were further analyzed to compare variations between egg averages and between end baking temperature averages.

Comparison of egg averages. Measurements of penetration (crust on sample), of curd tension, and of per cent sag indicated the average for custards made with dried egg differed significantly [d] from the averages of samples prepared with shell, homogenized frozen, and blended frozen eggs. No significant differences were found between shell, homogenized frozen, and blended frozen egg averages for these tests. Penetrometer measurements on samples with crust removed indicated the average gel strength of dried egg custards varied significantly[d] from that of samples prepared from shell and homogenized frozen eggs. The average gel strength of dried egg custards was significantly different from that of blended frozen egg custards at the 5% level of probability only. No significant differences were found between shell, homogenized frozen, and blended frozen egg averages.

Comparison of end baking temperature averages. Comparison of the end baking temperature averages in the penetrometer test for samples with crust on indicated custards baked to 90° C. were significantly different[d] in gel strength from those baked to 86 and 88° C. Differences in end baking temperature averages for custards baked to 86 and 88° C. were not significant. Penetrometer measurements on samples with the crust removed indicated custards baked to 88 and 90° C. were not significantly different from each other. Samples baked to 86° C. differed significantly from those baked to

[d] Studentized range test: 1% level of probability.

TABLE 1

Mean objective measurements on baked custards prepared from four types of whole egg and baked to three internal end temperatures

Objective tests	Temp. °C.	Type of egg					Least significant difference		
		Shell	Homo. froz.	Blend froz.	Dried	Mean		5%	1%
Penetrometer crust-on (mm.)	86	32.88	32.65	33.00	36.35	33.72	Egg	1.44	1.97
	88	30.95	32.48	32.35	34.93	32.68	Temp.	1.24	1.70
	90	29.45	30.35	30.65	32.88	30.83	Cell	2.49	3.41
	mean	31.09	31.83	32.00	34.72				
Penetrometer crust-off (mm.)	86	35.73	34.80	35.93	40.45	36.73	Egg	2.35	3.22
	88	31.93	33.80	34.68	36.30	34.18	Temp.	2.04	2.79
	90	31.28	33.55	32.48	35.95	33.32	Cell	4.07	5.58
	mean	32.98	34.05	34.36	37.57				
Sag (%)	86	50.83	33.63	46.33	67.70	49.62	Egg	10.66	14.61
	88	23.88	25.90	34.63	46.50	32.73	Temp.	9.23	12.65
	90	15.73	22.63	22.95	44.03	26.34	Cell	13.05	17.88
	mean	30.15	27.39	34.64	52.74				
Curd tension (g.)	86	22.50	27.50	21.88	5.63	19.38	Egg	6.25	8.51
	88	37.50	26.25	25.63	20.63	27.50	Temp.	5.41	7.37
	90	40.63	38.13	40.63	28.13	36.88	Cell	10.83	14.74
	mean	33.54	30.63	29.38	18.13				

88° C. at the 5% level of probability and from those baked to 90° C. at the 1% level of probability.

Analysis of end baking temperature averages for per cent sag showed samples baked to 88 and 90° C. were not significantly different from each other but they did vary significantly[d] from samples baked to 86° C.

Comparison of internal end temperature averages in the curd tension test revealed that each increment in internal end temperature resulted in a significant increase[d] in curd tension.

On the basis of penetrometer readings, shell, homogenized frozen, and blended frozen eggs produced custards similar in firmness at each given end baking temperature. These custards were much firmer than dried egg custards baked to the comparable internal temperature. It appeared dried egg custards baked to 90° C. were comparable in firmness to shell and frozen egg custards baked to 86° C. This observation was in agreement with findings by Lowe *(7)* and by McBride *(8)*.

Differences between mean penetrometer measurements for samples with and without crust were studied. It was thought this comparison might provide a possible measurement of resistance due to crust for each type of egg used. Differences between mean penetrometer measurements of these tests were compared with respect to type of egg and end baking temperature. No consistent trends were evident within eggs, within end baking temperatures, or between end baking temperature averages although a trend was apparent for differences between egg averages. These values were 1.89, 2.22, 2.36, and 2.86 mm. for custards made with shell, homogenized frozen, blended frozen, and whole dried eggs, respectively.

Gel strength, as measured by per cent sag, increased with each increment in internal baking temperature within each type of egg. It was evident dried egg custards required a higher internal end temperature for comparable gelation. Dried egg custards baked to 90° C. appeared similar in gel strength to custards made with shell or frozen eggs baked to 86° C.

From the curd tension readings it was apparent shell, homogenized frozen, and blended frozen egg custards were similar when baked to 86° C. and were much more resistant to cutting than dried egg custards baked to the same internal temperature. When baked to 88° C. frozen egg and dried egg custards appeared similar in curd tension whereas custards made with shell eggs seemed slightly firmer. This latter finding is in accord with a study reported by Jordan and Sisson *(5)* in which they found custards prepared with fresh eggs were firmer than custards made with dried eggs when baked to 87 and to 88° C. Samples made from shell, homogenized frozen, and blended frozen eggs displayed similar curd tension when baked to 90° C. Dried egg custards baked to 90° C. were much less resistant to cutting but appeared comparable to shell or frozen egg custards baked to 88° C.

In view of the evidence presented by each of the objective tests, it was concluded the gelation point of custards prepared with dried whole eggs was higher than that of custards made from either shell eggs or frozen eggs. End baking temperatures of 86 to 88° C. seemed to be most favorable for custards made with either shell or frozen eggs. The data of this study do not indicate that dried egg custards baked to 90° C. had achieved optimum gelation.

In the test for syneresis the drainage weight for many of the samples tested included custard particles resulting from insufficient product structure. This

TABLE 2

Mean subjective scores on baked custards prepared from four types of whole egg and baked to three internal end temperatures

Palatability factors [1]	Temp. °C.	Type of egg					Least significant difference		
		Shell	Homo. froz.	Blend froz.	Dried	Mean		5%	1%
Crust	86	4.35	4.10	4.33	1.70	3.62	Egg	0.43	0.66
	88	4.13	4.10	4.20	1.60	3.51	Temp.	0.38	0.57
	90	3.98	3.83	4.05	2.15	3.50	Cell	0.75	1.14
	mean	4.15	4.01	4.19	1.82				
Inside color	86	6.00	6.10	6.15	5.45	5.93	Egg	0.07	0.18
	88	6.00	6.10	6.00	5.20	5.83	Temp.	0.10	0.14
	90	6.05	6.10	6.15	5.50	5.95	Cell	0.20	0.27
	mean	6.02	6.10	6.10	5.38				
Aroma	86	5.95	5.83	6.00	3.70	5.37	Egg	0.19	0.26
	88	5.83	5.70	5.83	3.30	5.17	Temp.	0.17	0.22
	90	6.05	5.98	5.83	3.60	5.37	Cell	0.33	0.44
	mean	5.94	5.84	5.89	3.53				
Texture	86	5.83	6.38	6.13	3.60	5.49	Egg	0.92	1.39
	88	5.50	6.33	6.60	3.58	5.50	Temp.	0.79	1.20
	90	5.10	5.85	6.30	4.63	5.47	Cell	1.59	2.41
	mean	5.48	6.19	6.34	3.94				
Consistency	86	5.13	5.70	5.30	2.68	4.70	Egg	0.64	0.87
	88	5.70	5.88	5.70	3.48	5.19	Temp.	0.55	0.75
	90	5.65	5.55	6.03	4.25	5.37	Cell	1.10	1.51
	mean	5.49	5.71	5.68	3.47				
Flavor	86	6.30	6.30	6.45	1.73	5.20	Egg	0.26	0.36
	88	5.90	5.93	6.20	1.70	4.93	Temp.	0.23	0.31
	90	6.13	6.15	6.23	2.13	5.16	Cell	0.45	0.62
	mean	6.11	6.13	6.29	1.85				

[1] Highest possible score = 7.

condition was more prevalent for samples baked to 86 and 88° C. than for those baked to 90° C. These data did not represent true measurements of syneresis and have been omitted from this report.

The relation between the pH of the custard mix and the cooked custard was studied. In all cases the cooked custards were more alkaline than the custard mix. This result is in accord with the findings reported by Lowe *(7)* and by Logue *(6)*. An analysis of variance of the changes in pH brought about by baking were not significant at the 5% level of probability for any type of egg.

Subjective evaluation. The cold custards, scored by the same panel throughout the study, were evaluated for quality of crust, inside color, aroma, texture, consistency, and flavor. Each factor was scored from 1 to 7, with 7 as the highest score. In addition the judges checked the most appropriate descriptive terms listed for each characteristic and/or included additional comments in the space provided.

Numerical scores, averaged with respect to type of egg and internal end temperature, are summarized in Table 2. The mean scores for each factor were statistically treated by analysis of variance. These analyses showed differences attributable to the type of egg were significant at the 1% probability level for all factors. Differences in mean scores due to end baking temperature were significant at the 5% probability level for inside color and aroma but were not significant for the remaining palatability factors. It seemed likely that the relatively low palatability scores for *aroma* and *inside color* were affected, in part, by the apparent incomplete rehydration of the egg solids which result in an undesirable concentration of egg particles at the surface of the baked sample.

For all characteristics, comparison of egg averages indicated dried egg custards were scored significantly lower[d] than custards prepared with shell, homogenized frozen, and blended frozen eggs. Differences between egg averages for shell, homogenized frozen, and blended frozen egg custards were not significant.

The evaluation of mean aroma scores for internal end baking temperatures showed custards baked to 88° C. were significantly lower[e] than those baked to 86 and 90° C. Comparison of differences between end temperature averages for mean inside color scores indicated custards baked to 88° C. were significantly different[e] from but only slightly lower than samples baked to 86 and 90° C. This difference (0.01 unit) was so small that it was considered negligible. No significant differences were found between end temperature averages for either aroma or inside color for custards baked to 86 and 90° C.

Crust. The crusts of dried egg custards, irrespective of end baking temperature, were different in character from the crust of products made with the other types of whole egg. They appeared much brighter in color, tender but thick, and were described as porous, grainy, and mealy in texture. It was observed that egg particles suspended in the custard mix tended to rise slowly to the surface when mixing was discontinued. It seemed likely this condition prevailed during the heating process until the gelation temperature was reached and contributed substantially to the intensity of color and thickness of

[e] Studentized range test: 5% level of probability.

crust in the finished product. Incomplete rehydration of the egg solids seemed apparent in the texture of the crusts. It was concluded a slightly longer rehydration period for the egg solids, before the addition of the remaining ingredients, might possibly reduce the thickness of the crust and improve the texture.

The baking procedure did not produce browning for any of the samples regardless of the type of egg used or the length of the baking period. Scores for crust were a composite evaluation of color and tenderness. All products were frequently described as "off-color." Lack of browning may have influenced the evaluation of crust more for shell and frozen egg custards than for dried egg custards. Panel comments indicated homogenized frozen egg custards had slightly tougher crusts than those of shell and blended frozen egg custards. As the internal baking temperature increased, the shell, homogenized frozen, and blended frozen egg custard crusts seemed to decrease in tenderness. Increased internal temperature for dried egg custards did not appear to alter the tenderness of crust.

Inside color. All samples prepared with shell, homogenized frozen, and blended frozen eggs were judged to be typical in color and were rated similarly for all internal end baking temperatures. Approximately 17% of the inside color judgments for dried egg custards were noted as "off color." It seemed feasible to conclude that the concentration of egg particles at the surface of the sample would affect the intensity of color within the sample. In some dried egg custards the inside color appeared uneven. The formation of gel structure during the baking process probably prevented some coalesced egg particles from reaching the surface of the sample.

Aroma. The majority of judgments for this factor indicated custards made with shell and frozen eggs were typical in aroma and comparable in acceptability when baked 86, 88, and 90° C. The aroma of dried egg custards was judged unsatisfactory at all end baking temperatures. The descriptive terms "off-aroma," overheated, uncooked, and "eggy" appeared frequently on the score cards.

Texture. Shell egg custards baked to 86° C. were judged smoothest; porousness increased as the end baking temperatures were raised. Increases in porousness were accompanied by a slight degree of syneresis. Samples made with homogenized frozen and blended frozen eggs were judged smoothest when baked to 88° C. A few samples made with frozen eggs became slightly porous when baked to 90° C. Because these changes occurred in such a small percentage of the samples tested, they were not considered representative of the series.

Texture judgments of dried egg custards indicated these gels appeared smooth in many samples but were not as glossy as the gels of custards made with the other types of whole egg. More than half of the samples were considered smooth at all end baking temperatures, but many samples were described as porous and curdled. Slight syneresis, noted for some samples at all end baking temperatures, was observed less frequently with increased internal end temperature.

Consistency. For shell egg custards, the majority of the judges preferred the consistency of samples baked to 88 and 90° C. over that of samples baked to 86° C. Only 25% of the judgments indicated these custards were too firm when baked to these temperatures. Homogenized frozen egg custards were

judged typical in consistency at all internal baking temperatures. Just a few judgments designated samples baked to 90° C. as too firm. The ratings for consistency in custards made with blended frozen eggs revealed samples baked to 86° C. were not firm enough. Those baked to 88 and 90° C. were noted as typical in firmness except for a very small percentage which were judged too firm when baked to 90° C. Judgments for the consistency of dried egg custards were nearly unanimous. Samples baked to 86 and 88° C. were far too soft. Custards baked to 90° C. improved in firmness but only 16% of these judgments indicated the samples had reached desirable consistency.

The statistical analysis of these scores indicated differences in consistency, attributable to the type of egg and end baking temperature, were not significant for shell, homogenized frozen, and blended frozen egg custards. The variation in descriptive terms noted by the judges may have been influenced to a certain extent by personal preference.

Flavor. In general, custards made with shell eggs and frozen eggs were consistently scored as typical in flavor for all end baking temperatures. Summarization of the descriptive terms for flavor for dried egg custards indicated only one sample in the entire series was judged as typical. All other samples were described as "off-flavor," uncooked, overheated, and "eggy." These observations parallel those recorded for the factor of aroma. These findings are in sharp disagreement with those reported by Dawson *et al. (3)*, Jordan and Sisson *(5)*, and McBride *(8)*. However, they do substantiate the findings of Termohlen, Warren, and Warren *(10)* and Bennion, Hawthorne, and Bate-Smith *(2)*.

SUMMARY

The gelation properties and palatability of shell eggs, homogenized frozen eggs, blended frozen eggs, and reconstituted spray-dried whole egg solids in standard custard baked to internal temperatures of 86, 88, and 90° C. were compared.

Within the limitations of this study, objective and subjective measurements showed an internal temperature of 86 to 88° C. produced optimum gelation in shell, homogenized frozen, and blended frozen egg custards. With the formula used, it was apparent dried egg custards baked to the same internal temperature were not comparable in firmness to that of custards made with shell or frozen eggs.

Subjective evaluations of quality of crust, inside color, aroma, and flavor indicated differences between custards made with shell and frozen eggs were not significant. For these factors, custards made with egg solids were significantly different from and somewhat less desirable than custards made from shell and from frozen eggs. These results indicate the need for additional study of the concentration of solids, mixing procedure and the end baking temperature of custards made with egg solids.

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SOME FACTORS AFFECTING THE STABILITY OF FROZEN WHITE SAUCES[a, b]

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The problem of liquid separation from precooked frozen foods thickened by flours or starches has received considerable attention in recent years because of the increasing commercial and home preparation of foods of this type. Hanson, Campbell and Lineweaver *(3)* found that the thickening agent is directly involved in this separation. After examining a large number of starches and flours in white sauce formulas in an attempt to find a practical solution to the problem, they found that waxy rice flour yielded white sauces with stability superior to those from any of the other thickening agents tested. Improved stability of frozen puddings was also observed when waxy rice flour was used *(4)*.

Other waxy starches and flours formed products which showed liquid separation after thawing but appeared to be reconstituted by heating. It was concluded *(3)* that the instability was caused by retrogradation of the starch and that the superior behavior of waxy rice flour over the other waxy starches and flours was a result of the somewhat shorter average branch length of this starch reported by Meyer and Fuld *(10)*.

The instability of starch pastes was studied as early as 1844, when Scharling *(13)* reported that freezing and thawing of a starch-containing system resulted in a well defined separation into a clear solution and an insoluble spongy material. Numerous recent discussions of starch retrogradation *(1, 2, 15)* have reviewed later work on this problem. Work reported in this paper was undertaken as an attempt to define further the causes of the instability of starch-thickened products.

The reported differences *(3)* in stability between white sauces prepared with waxy rice flour and those prepared with waxy rice starch suggested that factors other than the type of starch influence stability. Since the proteins and salts of milk, as well as those in flours, might affect the results, starch-water pastes as well as white sauces were prepared from all starches studied, and wheat and waxy rice flours were included for comparison.

Retrogradation appears to be a purely physical phenomenon brought about by association of segments of the starch molecules through intermolecular forces *(15)*. This association occurs most rapidly at temperatures near 0° C., and therefore results from freezing and thawing most starch pastes. Molecules of amylopectin, the sole constituent of the waxy starches, associate only with difficulty because of their branched nature, and the

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precipitates formed may be dispersed by heating them in water above 50° C. The use of cross-linked waxy starches in water pastes and in a white sauce formula was investigated in this study to learn if the cross-linkages would further interfere with retrogradation to such an extent as to make these modified starches useful in the production of frozen starch-thickened foods. Since the association of the branched molecules can presumably occur only through the outer branches, control of granular swelling and fragmentation by means of cross-linkages appeared to be a possible means of minimizing the availability of the branches for association.

The periodate oxidation method for determination of end groups of polysaccharides indicated that the number of glucose residues per end group in the waxy rice starch *(5)* used in the study was 20, very close to the values of 22, 23, and 25 previously found *(12)* by the same method for amylopectins from sago, tapioca and corn starches, respectively. Comparison of the stability of starch-water pastes and of white sauces prepared with these amylopectins offered a possible means of determining the importance of the average number of glucose residues per end group of amylopectin in freeze-thaw stability. Use of waxy starches, composed of only amylopectin in granular form, as well as nongranular amylopectins, offered a means of observing the importance of granular structure to stability. Although glycogen, a D-glucose polymer similar to amylopectin except for its more highly branched structure *(8)*, was known to be stable toward retrogradation in aqueous solution *(14)*, its behavior in a white sauce formula was checked for purposes of comparison.

EXPERIMENTAL METHODS

Materials. The flours, whole starches, and glycogen used were commercially available products with the exception of waxy rice starch, which was prepared in the laboratory by Dr. S. A. Watson of the Corn Products Refining Company from the same lot of waxy rice flour that was used in this study. The amylopectins were prepared by Dr. T. J. Schoch of the same company. A commercial hydrogenated vegetable shortening and sodium chloride (C. P.) were used. The skim milk used was processed under carefully controlled conditions by the Michigan State Creamery. That used for the white sauces of Series 1 was obtained in 4 lots. Sauces of Series 2 were prepared from a single lot of milk.

Preparation of Series 1 samples of white sauces and starch pastes. The amount of starch or flour (Table 1) was predetermined experimentally to give a sauce with a final viscosity of 30 g.-cm., measured on a Corn Industries Viscometer. The starch or flour was blended with 50 g. of shortening which had been liquified by slight warming. The skim milk, 1082 g., and 7.2 g. sodium chloride were added. The mixture was stirred until smooth and poured into the cooking beaker of a Corn Industries Viscometer *(6)* surrounded by a glycerol-water bath at $100 \pm 0.5°$ C. Cooking was continued 5 minutes after maximum viscosity was reached or, with those thickening agents showing no maximum viscosity, until the increase in viscosity was very small, usually about 10 minutes after the initial increase in viscosity had been recorded.

Samples weighing 120 g. were sealed in lacquered 5-Z short tin cans immediately. Eight samples were prepared from each batch. The samples were placed overnight in the quick-freeze section of a chest-type home freezer and then transferred to the storage section maintained at $-20.0 \pm 1.0°$ C.

Seven batches of sauce were prepared with each of the 10 starches and flours, with the preparation pattern consisting of 7 randomized blocks.

One batch of starch- or flour-water paste was prepared with each of the thickening agents listed in Table 1. The weight of each starch or flour was the same as that used in the white sauce, but 1082 g. of distilled water was substituted for the skim milk and the salt and shortening were omitted. Since viscosity of the pastes was too low to be

registered by the viscometer, a temperature of 95° C. within the paste was used as the end-point. The pastes were weighed into tins, frozen, stored and thawed as described for the white sauces.

Preparation of Series 2 samples of white sauces and starch pastes. In the preparation of small batches of white sauces with corn starch, waxy corn starch, phosphate cross-linked waxy corn starch, waxy rice starch and waxy rice flour (Table 3) the quantities of all the ingredients were 5% of those used for the batches in Series 1. The weight of 2 g. for glycogen and each of the amylopectins was arbitrarily chosen to give pastes of approximately the same concentration as the other samples, although of much lower viscosity.

The ingredients were mixed in a large test tube (200 mm. × 38 mm.) and heated to 95° C. in the water bath with only enough stirring to keep the starch suspended until it was gelatinized. Samples of 20 g. were weighed into porcelain crucibles, which were then wrapped in Saran film and frozen in the same manner as the samples of Series 1. Duplicate batches were prepared with each thickening agent.

Pastes were prepared in the same way with the thickening agents and distilled water. Controls of skim milk and of skim milk plus shortening and sodium chloride were heated to 95° C. and frozen.

Objective evaluation of Series 1 samples. Samples in the sealed cans were placed in a water bath at 35 ± 1° C. for one hour. Measurement of the amount of separated liquid was obtained by centrifugation of the thawed samples in a Servall Superspeed Angle Centrifuge at 6100 r.p.m. (about 12,200 x G.) for 15 minutes. The supernatant liquid was decanted and measured in a graduated cylinder.

Turbidity of the supernatant was measured by diluting a 1-ml. aliquot to 10 ml. with distilled water and measuring light transmission in a Bausch and Lomb Spectronic 20 colorimeter at a wave length of 625 mμ, chosen to eliminate effects of the yellow-green color of some of the liquids examined. The instrument was set to give a transmission of 100% with distilled water.

Liquid separation and turbidity were measured on samples from 2 batches of each white sauce after the periods of storage shown in Table 1. In the few cases in which the difference between the values for the 2 samples was greater than 10%, a third sample was tested.

Nitrogen content of the supernatant from samples stored 1 month was determined by the Kjeldahl method. The percentages of protein were calculated by multiplying the values for nitrogen by 6.38, the factor given for milk by the Association of Official Agricultural Chemists *(10)*.

Additional samples were examined for reconstitution properties by heating them in the sealed cans in a boiling water bath until the temperature of the sauce reached approximately 90° C. Tests for liquid separation and turbidity were conducted as described above.

Subjective examination of Series 1 samples. Thawed samples were evaluated by a panel of 5 judges for appearance of separation, smoothness, general acceptability and other subjective characteristics. Four replications were presented after each storage time indicated in Table 2. Samples were grouped so that each of the thickening agents was presented with each of the others at least once, thus allowing all possible comparisons. Five thawed samples and a freshly prepared standard white sauce were presented to the panel at each session. A 7-point scoring scale was used, the higher scores indicating more acceptable products.

Objective evaluation of Series 2 samples. Samples were allowed to stand in the laboratory for 2 hours to bring them to approximately room temperature. Tests for liquid separation and turbidity, conducted after the storage periods noted in Table 3, were the same as those used for the samples of Series 1.

RESULTS AND DISCUSSION

From samples prepared with wheat flour, ordinary corn and sorghum starches, and waxy corn and sorghum starches much clear watery liquid separated upon thawing after holding in frozen storage for one week (Table 1). Little further change occurred when the storage time was extended to one month. Lumps in the sauces from the waxy starches were slippery and

TABLE 1

Liquid separation from Series 1 samples

Thickening agent	Weight	Separated liquid (% total weight)						Supernatant: Clarity (% light transmission [1])						Supernatant: Protein content (%)
		Pastes	Sauces					Pastes	Sauces					Sauces
Storage time		1 wk.	1 wk.	1 mo.	3 mo.	6 mo.		1 wk.	1 wk.	1 mo.	3 mo.	6 mo.		1 mo.
Thawing temperature		35° C.	35° C.	35° C.	35° C.	35° C.	100° C.	35° C.	35° C.	35° C.	35° C.	35° C.	100° C.	35° C.
	(g.)													
Wheat flour	58.0	37	39	47	46	48	35	94	88	88	89	98	82	0.62 (0.41) [2]
Waxy rice flour	37.5	61 [3]	0	0	17 [3]	27 [3]	17 [3]	66	—	—	82 [4]	74	53	—
Waxy rice starch	21.5	44	22	25	29	37	19	94	54	72	85	97	90	1.16
Waxy corn starch	21.1	68	40	40	52	62	12	96	85	84	98	98	83	0.86
Waxy sorghum starch	22.0	73	41	42	50	65	8	94	90	87	94	99	77	0.75
Corn starch	40.0	33	50	50	57	55	42	96	83	84	90	98	96	0.61
Sorghum starch	38.0	32	47	51	42	53	41	96	84	87	95	98	94	0.54
Cross-linked waxy corn starch	41.5	57	39	45	51	54	31	94	79 [4]	81 [4]	88	93	91	1.90
Phosphate cross-linked waxy corn starch	36.1	62	40	45	46	61	33	96	88 [4]	82 [4]	90	98	90	1.72
Phosphate cross-linked waxy sorghum starch	37.0	62	41	51	59	62	26	97	84 [4]	58	92	99	93	1.45

[1] Distilled water = 100% transmission. Samples diluted 1:10 unless marked (4).
[2] Value for soluble protein of flour subtracted.
[3] Viscous supernatant.
[4] Samples diluted 1:100.

TABLE 2

Panel scores for Series 1 sauces

Storage time	Smoothness			Lack of separation			General acceptability		
	1 wk.	1 mo.	3 mo.	1 wk.	1 mo.	3 mo.	1 wk.	1 mo.	3 mo.
Control (wheat flour, freshly prepared)	6.0	6.7	6.8	6.5	6.8	6.8	5.7	6.3	6.5
Frozen samples									
Wheat flour	1.9	2.1	1.4	2.3	1.9	1.5	1.7	2.2	1.6
Waxy rice flour	6.2	6.2	6.1	6.7	6.5	6.0	5.3	4.6	4.9
Waxy rice starch	4.4	3.9	3.0	5.5	4.3	3.4	3.6	3.0	2.3
Waxy corn starch	3.3	2.7	2.0	3.7	2.7	1.7	3.3	2.7	1.8
Waxy sorghum starch	3.3	2.6	1.9	3.4	2.1	1.3	3.2	2.4	1.8
Corn starch	2.4	2.1	1.4	1.3	1.2	1.0	1.3	1.3	1.1
Sorghum starch	2.3	2.2	1.5	1.2	1.2	1.1	1.4	1.3	1.1
Cross-linked waxy corn starch	4.2	3.2	1.4	5.3	3.8	1.5	3.2	2.8	1.5
Phosphate cross-linked waxy corn starch	4.3	3.2	2.1	5.6	4.6	2.6	3.5	2.6	1.8
Phosphate cross-linked waxy sorghum starch	5.2	2.3	1.4	5.7	3.2	1.1	5.0	2.3	1.2

[1] Maximum possible score, 7.

TABLE 3

Liquid separation from Series 2 samples

Thickening agent	Weight	Separated liquid (% total weight)				Clarity of supernatant (% light transmission [1])			
		Pastes		Sauces		Pastes		Sauces	
Storage time		1 wk.	3 mo.	1 wk.	3 mo.	1 wk.	3 mo.	1 wk.	3 mo.
	(g.)								
Corn starch	2.00	38	39	49	58	94	100	91	98
Waxy corn starch	1.06	75	79	26	55	96	99	97	99
Phosphate cross-linked waxy corn starch	1.80	44	44	36	55	98	100	80 [2]	99
Waxy rice starch	1.08	44	54	21	35	99	99	70	98
Waxy rice flour	1.81	39 [3]	47 [3]	0	29 [3]	89	95	—	92
Sago starch amylopectin (S-2-B) [4]	2.00	85	89	48	60	69	92	72	96
Waxy corn starch amylopectin (WM-1/2-B) [4]	2.00	59	67	38	34	69	86	83	87
Tapioca starch amylopectin (T-8-B) [4]	2.00	0	0	0	43 [3]	—	—	—	87
Glycogen	2.00	0	0	0	88	—	—	—	72
Controls									
Skim milk	—	—	—	0	82	—	—	—	91
Skim milk with fat and sodium chloride	—	—	—	0	88	—	—	—	72

[1] Distilled water = 100% transmission. Samples diluted 1:10 unless marked (2).
[2] Samples diluted 1:100.
[3] Viscous supernatant.
[4] For preparation, see Reference *(7)*.

stringy, rather than sandy and friable as in the sauces from the ordinary varieties of starch or from wheat flour, but they were only slightly less objectionable to the panel of judges (Table 2). When the sauces from waxy starches were heated after a 6-month storage period, the separation disappeared to a considerable degree, but the undesirable texture characteristic of unmodified waxy starches persisted.

The similar behavior of starch-water pastes prepared from the same starches supported the report of Hanson and co-workers *(3)* that the thickening agents are a major factor in the instability of frozen white sauces. However, when those sauces thickened with waxy starches were heated in a water bath at 100° C., complete reversal of the separation, as reported by the previous investigators, was not observed, though such reversal would be expected were the lump formation caused entirely by retrogradation of the amylopectin. These samples, although greatly improved by heating, still contained some large, soft lumps, and some liquid separated upon centrifugation (Table 1).

Phosphate cross-linked starches, particularly a phosphate cross-linked waxy sorghum starch, yielded sauces which were considered by the panel of judges to be more acceptable in several respects after one-week storage than sauces from wheat flour, regular corn or sorghum starch, or unmodified waxy corn or sorghum starch (Table 2). However, the amount of liquid separated during centrifugation was approximately the same as for the starches judged less acceptable. The difference in acceptability apparently was caused by the far greater turbidity of liquid separated from the sauces thickened with the cross-linked starches (Table 1), as well as the smaller, softer lumps. Turbidity was appreciably less after one month than after one week, although greater than that for the other starches. Nitrogen analysis of supernatants from samples stored one month showed more of the milk protein was retained in the liquid separated from the cross-linked starches than in supernatant liquids from unmodified starches. However, the volumes of separated liquid had increased over those from samples stored for shorter times, and the appearance and acceptability to the judges was reduced to a low value. The cross-linkages apparently did not prevent retrogradation, although they seem to have retarded the precipitation of protein from the milk.

Subjective scores on the sauce from waxy rice starch approximated those for the sauces from the cross-linked starches after shorter storage periods (Table 2), possibly because the liquid separated from the waxy rice starch, although less in volume, was more clear. Scores for these samples were somewhat higher than those for the unmodified waxy starches.

Sauces prepared with waxy rice flour were not separated by centrifugation after one-month storage. Some separation was found by centrifugation after 3 months, although it was not sufficient to be observed by the scoring panel. The supernatant liquid was viscous and difficult to separate from the sediment. The reason for the lower score given by the judges for general acceptability, despite their high scores for smoothness and lack of separation, may have been caused by the flavor of the waxy rice flour, described by some as typical of raw cereal. Possibly this flavor could have been avoided by a lower cooking temperature, since it has been reported that an unpleasant foreign taste is developed if a product containing waxy

rice flour is heated above 190° F. (87.8° C.) *(9)*. However, lower cooking temperature would have necessitated use of an increased amount of the flour. Another factor causing the lower score may have been the slippery quality of the waxy rice flour sauce.

The pronounced difference in behavior of waxy rice flour and waxy rice starch indicated that the stability of the former was not entirely the result of the characteristics of the starch constituent, although this starch provided better stability than any other unfractionated starch examined. Other constituents of the flour, possibly protein and/or salts, appeared to enhance this property of the starch. Stability of both waxy rice starch and waxy rice flour was better in a white sauce formula than in water.

Results for waxy corn amlyopectin (nongranular), waxy corn starch, and phosphate cross-linked waxy corn starch in the samples of Series 2 indicated that stability to freezing and thawing was influenced very little by the presence or absence of granular structure.

The small amount of precipitate which formed when glycogen was used in a white sauce formula and stored frozen for 3 months was identical with that from a control sample containing the other ingredients of the white sauce formula without a thickener. Thus the high degree of branching in the glycogen molecules appeared to prevent retrogradation in the white sauce formula as well as in water. However, water solutions of amylopectins from sago and waxy corn starch were highly unstable to freeze-thaw treatment, although a similar solution prepared from the amylopectin of tapioca starch was completely stable after 3 months' frozen storage. When tapioca amylopectin was used in a white sauce formula, no separation had occurred after one week of storage, thus indicating stability to freeze-thaw treatment equaled only by waxy rice flour among those thickening agents tested. With both tapioca amylopectin and waxy rice flour, the liquid which separated upon centrifugation after 3 months of storage differed from the other supernatants in being very viscous. This stability of products from tapioca amylopectin indicated that structural differences within amylopectin molecules may exert a very considerable effect upon stability toward freeze-thaw treatment. The nature of these structural differences is not evident at present.

The sago and tapioca amylopectins and the waxy rice starch used in this study were the identical preparations for which the number of glucose residues per end group had been found to be 22, 23 and 20, respectively, by the periodate method *(5, 12)*. The similarity of the values, plus the fact that the stable tapioca amylopectin had an equal or higher average number of glucose units per end group than the unstable sago amylopectin, appeared to be in contradiction to the theory *(3)* that the superior stability of waxy rice flour is related to the lower number of glucose units per end group of its starch. Length of the outer branches of the amylopectin molecules, however, does not seem to be ruled out as a possible factor. Analyses employing enzyme techniques have indicated that the average outer branch lengths of amylopectins may differ appreciably *(8)*. Even data of this type, however, fail to show whether the outer branches are all of about equal length within a molecule, a condition which might influence tendency toward retrogradation. It appears that more complete information concerning fine points of the structure of amylopectins may be necessary before causes of

the differences in their tendencies toward retrogradation and, consequently, freeze-thaw stability can be elucidated.

The effect of constituents of foods other than starch on freeze-thaw stability was indicated above by differences in behavior between waxy rice flour and starch prepared from it. Further evidence of the importance of other ingredients lay in the differences in stability between starch-water pastes and white sauces prepared from the same thickening agent. In general, the white sauces seemed somewhat more stable than the corresponding pastes, although the reverse was true for tapioca amylopectin. Thus, although the nature of the starch is probably the most important factor in determining the stability of a starch-thickened product, it appears that the effects of other ingredients are also of considerable importance.

SUMMARY

Instability of starch-water pastes, as well as white sauces prepared from the same starches and flours, indicated that retrogradation of starch was probably the major factor affecting stability. However, the rate of retrogradation varied greatly with different starches and starch fractions. Pastes and sauces prepared from amylopectins from different starches varied greatly in their stability. Tapioca amylopectin and waxy rice flour produced white sauces of about the same stability, greater than that of any other thickening agent tested.

Constituents of milk and of flour other than starch appeared also to exert appreciable effects on stability. Pastes of tapioca amylopectin and water alone were much more stable than white sauces containing the same starch fraction or than pastes of waxy rice flour. White sauces containing waxy rice flour were more stable than those containing waxy rice starch.

Both the structure of the starch used and the other ingredients present were found to be important in determining the freeze-thaw stability of a starch-thickened food.

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APPLICATIONS OF NATURAL GAMMA ACTIVITY MEASUREMENTS TO MEAT*

The principal gamma radioactivity observed in living things is due to the naturally radioactive isotope of potassium, K-40. This nuclide has a half-life of 1.3×10^9 years and constitutes 0.01% of the natural mixture of potassium isotopes. The other stable isotopes of potassium have mass numbers 39 and 41. Isotopic fractionation effects are very small because of the small percentage mass differences, so that all potassium has essentially the same K-40 content and hence, the same specific radioactivity. A determination of the K-40 activity is therefore equivalent to a determination of total potassium.

K-40 decays 90% of the time to Ca-40 with the emission of a negative beta ray. Ten per cent of the time it captures an orbital electron, becoming A-40 and emitting a gamma ray of 1.45 Mev energy. The natural mixture of potassium isotopes emits 2.96 gamma rays per second per gram *(2)*. The absolute disintegration rate does not enter into the calibration of the system, however, since one obtains a direct calibration of the detector in terms of observed counting rate per gram of a potassium standard (e.g., KCl). The calibration factor then includes the product of the absolute gamma emission rate and the absolute efficiency of the counter, neither of which needs to be known to as high a precision as can be obtained on unknowns. It is, of course, important that the calibration be performed on a standard which is identical to the unknowns in all important parameters such as average atomic number, total mass, density, and shape.

Since the concentration of potassium in living cells is held constant by homeostatic processes, a determination of potassium content is equivalent to a determination of cellular mass. There is no potassium in fat and very little in bone. From the determination of total potassium content, it has been shown to be possible to estimate the "lean-body" or "fat-free" mass of man *(3)* and of hams *(1)*. Applications to the meat industry are based on this proportionality between potassium and mass of lean tissue. Clearly, no information is obtained as to the identity of the "non-lean" portion of the sample; it could be fat or bone, or other inert material.

ADVANTAGES OF THE COUNTING METHOD

The outstanding advantage of the counting method over chemical analysis is its adaptability to continuous systems. If the sample to be analyzed (e.g., comminuted meat) can be passed through the counter in a continuous stream of constant bulk density, the counter will provide through a rate-meter a continuous measurement of the lean content of the meat. The use of such a system for control of the ratio of lean/fat input to the grinders is obvious. Whether or not such a system is feasible depends on the precision and response time requirements.

Precision and response time of the counter are directly related once sample size is specified. For a given sample size, the time required increases

* Dr. E. C. Anderson, author of this note, has been a consultant of Radiation Counter Laboratories, 5121 W. Grove Street, Skokie, Illinois, for the development of an instrument to detect and measure extremely low activities in foods.

as the inverse square of the precision (e.g., to obtain 1% precision requires a four-fold slower response time over 2% precision). The response time is determined by the integrating time over which the rate-meter circuit averages the counting rate. Since the standard deviation of any counting determination is given by the square root of the total number of counts observed, one must observe 4 times as many counts to reduce the relative error by a factor of 2. Figure 1 is a graph of the time required to obtain a given statistical precision for various sample sizes. The conditions assumed here are essentially those of the ham study *(1)* i.e., one count per second per pound of meat. While the lean content of a comminuted product may be lower than that of the ham, counter efficiency can be increased in an improved design. The counter is also assumed to be of optimum size for the sample.

For errors of not less than 2%, experience indicates that counting statistics will indeed be the limiting factor. Large counters at the Los Alamos Scientific Laboratory have been operated with a precision of 0.5% for K-40 in people and foods. However, the possible influence of other factors will have to be evalulated if a precision of better than 2 per cent is desired. Clearly, the mass of sample in the counter at any time must remain constant to better than the desired precision level. The capability of the conveyor system to maintain this stability must be investigated. Another possible source of error is contamination of the meat with extraneous radioactivities (e.g., natural radium or fission products from fallout). They have not constituted a limiting factor in measurements at the 3% level, but there will be a level of precision at which they must be taken into account.

The useful time constant of the counting system cannot be considered apart from the system which it is controlling. The important parameters are the rate of flow of sample through the counter (i.e., the rate of production of sample by the machine) and the time lag for the sample material to flow from the point of injection into the machine until it reaches the counter. For example, if the counter is designed to contain 50 pounds of sample and the rate of production is only 5 pounds per minute, the counter will at any moment contain the entire production for 10 minutes of operating time. If the composition of the product were to change abruptly, 10 minutes would elapse before the contents of the counter were completely replaced with material of the new composition. The response time of such a system would be 10 minutes, regardless of the integrating time of the counter and rate-meter.

Similarly, if it were necessary to place the counter at such a point in the flow pattern that the product required 5 minutes to pass from the injection point to the counter, the control of the mixture could not be faster than the 5-minute delay.

For an illustrative design, we will assume that 2 per cent statistical precision is required ($\pm 2\%$ for the 2 sigma limits) and that the response time of the entire system must be 3 minutes. Referring to Figure 1, we see that $1\sigma = 1\%$ in 3 minutes requires a sample size of 100 pounds. The machine must therefore have an output of at least 33 pounds per minute, 2000 pounds per hour. Total holdup in the processing system between injection and counting must be less than 3 minutes of production so as

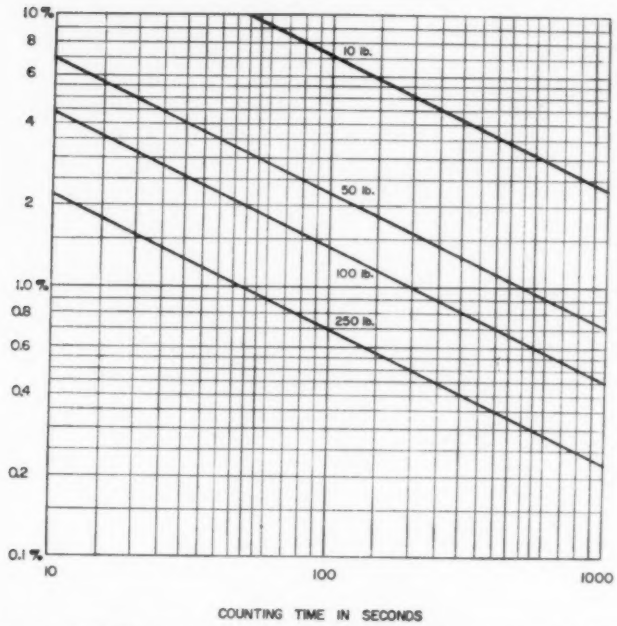

Figure 1. Statistical error in K^{40} determination in meat as a function of counting time for various sample sizes.

not to introduce a delay greater than the response time. It may be difficult to locate the counter this close to the injection point, since there must be sufficient mixing and grinding before the counter to ensure a homogenous material at the point of counting.

For batch operation the problems are simpler, but the gain over chemical analysis is less. Sample size can be accurately determined by weighing and does not constitute a possible source of error. The large sample size simplifies the problem of obtaining a representative aliquot. The analysis can be easily performed in a few minutes, far more rapidly than by chemical means. The principal question here is economic and can be answered only after a reliable cost estimate for the counting system is obtained.

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